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In vitro Micropropagation of *Rosa hybrid* L.

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Abstract

To determine the appropriate concentrations of nutrients and growth regulators for shoot proliferation and root initiation, several rose hybrid tea cultivars were cultured. Cultured shoot tips and lateral buds from different cultivars proliferated multiple shoots on Murashige and Skoog (MS) medium supplemented with 0 to 4 mg/L BA and 0 to 0.05 mg/L NAA. The ability of the explants to proliferate shoots and initiate roots was affected by genotype, the nodal position of explant, the strength of MS basal medium and growth regulators used. The buds nearest the apex exhibited the slowest rate of development. Most cultivars had the highest shoot proliferation when cultured on MS medium with 2 mg/L BA and 0.01 mg/L NAA, but the degree varied by cultivars. Root development was enhanced by lowering the concentration of MS salts.

Key words: Nodal position, shoot proliferation, rooting

Introduction

The rose is one of the most economically flowers in the world. There are more than 20,000 commercial cultivars, which collectively are based on only 8 of the approximately 200 wild species in *Rosa* (Roberts et al. 1990).

Micropropagation procedures have been improved by a number of researchers in four major groups of rose cultivars: hybrid teas (Dohare et al. 1991), floribundas (Douglas et al. 1989), miniatures (Rogers and Smith 1992; Chu et al. 1993), and Climbers (Davies 1980). Today, rose tissue cultures are exploited for various purposes, from basic anatomical and physiological research (Donelly and Skelton 1989; Korban and Donelly 1994) and extration of androgenetic haploids (Tabaeezadeh and Khosh-Khui 1981) to micropropagation from calli, immature embryos, or protoplasts (Burger et al. 1990; Roberts et al. 1990; Mattews et al. 1991; Rout et al. 1991). The macropropagation of 400,000 plants per year from a single rosebush is technically possible (Short and Roberts 1991).

Hormone concentrations, nutrients levels, temperature treatments, explants sources, and genotype have been shown to affect micropropagation in cultivated rose. While it is quite easy to proliferate shoot tips and axillary meristems of most cultivars, the rate of shoot proliferation varies considerably among cultivars (Skirvin et al. 1990; Short and Roberts 1991). In *R. persica*× *R. xanthina*, the required concentrations of benzyladenine (BA) and naphthalene acetic acid (NAA) for shoot induction were lower for calli obtained from shoots that had themselves been regenerated from callus culture, although the cytokinin was still required at about 20 times the concentration of the auxin (Lloyd et al. 1988). Some micropropagation media contain gibberellin, but it can reduce shoot survival and leaf expansion (Skirvin et al. 1990). Although it appears to be relatively easy to proliferate rose shoots *in vitro*, rooting is frequently difficult. Early research sought optimal concentration of auxins and cytokinins for good rooting (Jacob et al. 1970a,b). Vitamin D_2 augments induction of rooting by NAA, and rooting was more robust in perlite than agar (Skirvin et al. 1990). Sometime the concentration of entire suite of salts was reduced 50 to 75% to promote rooting (Short and Roberts 1991). Several researchers reported definite optimal inorganic salt concentrations, light intensities, photoperiods, and temperatures for shoot and root induction and growth (Hyndman et al. 1982a; Dorion et al. 1991). Bressan et al. (1982) also found that the 5 th through the 15 th nodes from the shoot apex were the most responsive to micropropagation, and that the rapidity of response was influenced by cytokinins.

The ability of induced shoots to photosynthesize is influ-

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enced by the sucrose concentration of the medium. Micropropagated shoots die if they are suddenly moved to medium without sugars, but too much sucrose represses photosynthetic capacity (Hyndman et al. 1982b; Lanford and Wainwright 1988). Photosynthetic competence is also influenced by light intensity and availability of carbon dioxide in the culture jars. Cuticular and stomatal function on induced shoots are affected by chemical growth retardants and relative humidity in culture (Roberts et al. 1992).

In this report, the effects of genotype, plant growth regulators, nodal postion on micropropagation of rose hybrid teas, which is the most important species for breeding of cut rose in the world, were investigated.

Materials and Methods

Plant materials and general procedures

Six commercial rose (*Rosa hybrida* L.) cultivars, '4th of July', 'Graham Thomas', 'Tournament of Roses', 'Sequoia Ruby', 'Little Artist' and 'Playboy' were used in this study. These hybrid tea cultivars were grown in an unshaded greenhouse. Culture media included MS basal salts (Murashige and Skoog 1962) supplemented with 2 mg/L glycine, 100 mg/L I-inositol, 0.4 mg/L thiamine, 0.5 mg/L pyridoxine, 0.5 mg/L nicotinic acid, 30 g/L sucrose, and 7 g/L Sigma agar. The pH of the medium was adjusted to 5.7 before adding the agar. Media were autoclaved for 20 min at 120°C. Cuttings of each genotype were collected from the greenhouse and surface sterilized for 2 min in 70% ethanol, followed by a 5 min soak in 20% bleach, and then one to three rinses with sterile water. Cultures were incubated under 24 hours of cool-white fluorescent light (20-30 mol/m^2/s^1) per day at 25°C. Each combination of genotype and treatment involved 20-25 explants per experiment (rarely 10-15), and each experiment was conducted at least twice.

Effect of nodal position and genotype

Stems from greenhouse-grown plants of each genotype were divided into an apical shoot cutting (0.5-1.0 cm) and up to 16 one-node cuttings (1-2 cm equal sections). Surface-sterilized explants were placed in 8-dram vials that contained 10 mL of full-strength MS media. After 20 days(d), the length of shoots, the number of roots, and the percentage of browning (explants that turned brown and failed to break bud dormancy) were determined for each genotype and nodal position.

Proliferation experiment

Developing shoots (about 1.5 cm long including the apex) were detached from previously cultured nodes and transferred to 50 mL of fresh medium per 300 mL culture vessel for shoot proliferation. There were five shoots per vessel. The shoot proliferation media contained full strength MS salts and various levels of BA (0, 1, 2, and 4 mg/L) and NAA (0, 0.01, and 0.05 mg/L). After 21-23 d, the number of new shoots at least 1 cm in length was counted.

Root initiation

For rooting, three concentrations of MS salts (full-, half-, and quarter-strength) were tested on shoots that had been regenerated from nodes. The effect of IAA supplementation at 1 mg/L on root initiation was also tested in half-strength MS. After 21 d, roots were counted and their lengths were measured.

Greenhouse acclimatization

Young rooted plants were gently dislodged from the agar medium and transplanted into a culture medium (2 parts pine-bark mulch: I part peat moss: 5 parts sand). The plants were grown under ambient daylight in an unshaded greenhouse with a day/night temperature regime of 25°C/22°C±5°C. The plants were covered with plastic domes for 10 d to maintain high humidity (70%). The percentage of suvival was recorded after the plants had grown in the greenhouse for 90 d.

Results and Discussion

Nodal position and genotype

Shoot and root growth from explants were affected by nodal position and genotype. In general, the least growth and highest mortality occurred for the nodes closest to the shoot apex (Table 1), as previously reported in rose cultivars (Bressan et al. 1982). The poor performance of the nodes nearest the apex possibly resulted from their smaller diameter and more herbaceous nature. Explants from nodes 7th to 16th exhibited relatively uniform growth. The cultivar, 'Tournament of Roses' showed the best response for shoot growth with little difference among nodes in shoot growth. However, cultivars Playboy and Little Artist showed less shoot growth than the other cultivars. Some explants, especially those from the shoot apex itself, released a soluble brown substance from the wound surface into the medium. The amount and frequency of browning of the shoot apical explants were inversely correlated with bud growth. The brown substance was possibly a ployphenol that is toxic to the explant (Skivin et al. 1990). Explants other than the apical shoot exuded less brown material when transferred to fresh medium after 3-5 d, possibly reflecting adaptation to the medi-

Table 1. Effect of the node position on shoot growth of rose species grown *in vitro* on full-strength basal Murashige and Skoog (MS) medium without growth regulator for 20 days

Cultivars	Shoot length (cm)					
	Tip	1-3 node	4-6 node	7-9 node	10-12 node	13-15 node
4th of July	4.2±1.2[a]	2.6±1.2	4.8±1.3	4.8±2.3	5.1±3.1	-[b]
Graham Thomas	2.5±0.7	2.7±0.8	3.3±1.1	3.5±1.6	-	-
Tournament of Roses	3.1±1.2	1.8±0.4	4.3±1.7	4.5±1.9	4.3±2.7	-
Sequoia Ruby	1.8±0.6	1.2±0.6	2.3±0.8	2.5±0.8	2.3±0.9	2.0±1.1
Little Artist	1.4±0.4	1.9±1.1	3.1±1.2	-	-	-
Playboy	0.0±0.0	1.2±0.5	2.7±1.1	3.7±1.6	3.4±1.6	3.2±1.9

[a]Data represent the mean±SE of shoot length.
[b]-means data not available.

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The genotypes differed greatly in rooting (Table 2). Only two cultivars, Tournament Roses and 4th of July, rooted without addition of growth regulators in media. The nodes closest to the apex rooted less. The high rooting from the shoot apex in 'Tournament Roses' was consistent with the vigorous growth of shoots and lack of browning from its cultured shoot apex.

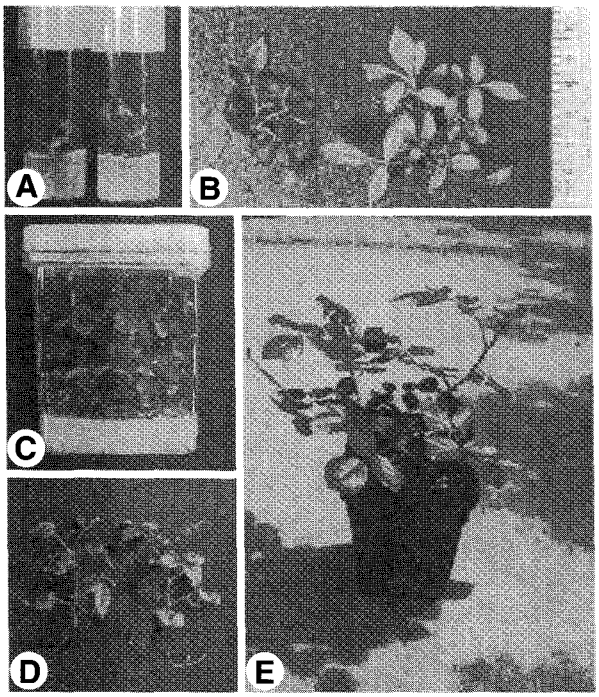

Figure 1. Plant regeneration via adventitious shoot formation from node culture of hybrid tea rose, A; Shoot growth from cultured shoot apex (left) and 5th node (right), B; Shoot proliferation from cultured shoot apex (left) and 5th node (right), C; Shoot development from adventitious shoot on MS medium with 2 mg/L BA and 0.01 mg/L NAA after one month, D; Root initiation on half-strength MS medium without plant growth regulators, E; Plantlet in greenhouse.

Shoot proliferation

Shoot proliferation was influenced by plant growth regulator levels and genotype. Low concentrations of BA stimulated development of axillary buds, but higher BA (4 mg/L) inhibited shoot proliferation. The highest shoot proliferation generally occurred with 2 mg/L BA, but genotypes varied greatly in BA responsiveness (Table 3). Shoots per explant at 2 mg/L BA ranged from about 10 ('Tournament Roses') to about 3 or less ('Sequoia Ruby'). The addition of NAA generally decreased shoot proliferation. The only exception was 'Little Artist', in which shoot production significantly increased (30%) with 0.01 mg/L NAA in addition to 2 mg/L BA.

Plant response during rooting treatment

Although rose shoots often proliferate readily *in vitro*, rooting those shoots was more difficult. Rooting was affected by genotype, MS medium salt concentration, responded better with low salts, cold dark treatment, and IAA supplementation (Table 4). Reduced salt concentration generally increased rooting in MS

Table 2. Effect of the position on *in vitro* root number of plantlets grown on half-strength basal MS medium without growth regulator for 20 days

Cultivars	Number of roots per explant			
	Tip	1-3 node	4-6 node	7-9 node
4th of July	2.5±0.8[a]	1.5±1.1	2.0±1.3	1.3±0.5
Graham Thomas	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Tournament of Roses	1.8±0.6	0.7±0.3	1.5±0.6	1.0±0.6
Sequoia Ruby	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Little Artist	0.0±0.0	0.0±0.0	0.0±0.0	-
Playboy	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

[a]Data represent the mean±SE of root formation per explant. Minimum root length counted was 0.5 cm.

Table 3. Shoot proliferation of rose species cultured 21 days in MS media supplemented with BA and NAA

Plant growth regulator (mg/L)		4th of July	Graham Thomas	Tournament of Roses	Sequoia Ruby	Little Artist	Playboy
BA	NAA						
0	0	3.4±1.7[a]	1.4±0.5	2.4±1.3	1.1±0.6	1.1±0.5	2.2±0.7
1	0	6.5±2.9	1.4±0.6	3.5±1.7	1.4±0.8	3.2±1.6	6.3±3.1
2	0	9.3±3.5	4.9±2.3	10.1±4.2	2.5±0.4	3.6±1.9	7.7±2.2
2	0.01	7.3±3.4	3.3±1.4	6.1±3.2	2.0±1.0	5.3±2.6	4.7±3.3
2	0.05	6.0±2.8	2.2±1.1	3.1±2.1	1.5±0.8	3.2±1.4	3.8±1.5
4	0	3.9±1.6	1.7±1.2	2.8±0.7	1.3±0.5	2.2±0.8	3.8±0.9

[a]Data represent the mean±SE of shoot formation per explant.
Length of shoot at least 1.0 cm.

Table 4. *The effect of MS salts on the root initiation of cultured rose shoots*

Cultivars	1/4 MS	1/2 MS	1/2 MS+1 mg/L IAA	MS
4th of July	6.6±2.2	3.2±1.3	-	3.1±1.4
Graham Thomas	4.5±1.9	1.1±0.5	1.3±0.6	0.0±0.0
Tournament of Roses	7.8±3.6	5.7±3.1	-	3.4±2.2
Sequoia Ruby	3.8±1.2	3.7±1.8	5.1±2.6	0.0±0.0
Little Artist	2.2±0.7	1.1±0.8	1.2±0.5	0.0±0.0
Playboy	4.9±2.5	1.8±0.6	3.9±1.9	0.0±0.0

[a]Data represent the mean of root formation per explant.
Minimum root length was 0.5 cm.

media, in accordance with experience in other species (Skirvin et al. 1990). The most roots were initiated per explant on quarter-strength MS medium.

The supplementation with 1 mg/L IAA significantly increased rooting over that obtained on half-strength MS medium. However, the IAA treatment was not consistently more effective or less effective than the other treatment. In this study, the survival of plants from different rooting treatments ranged from 75 to 90% in the greenhouse.

In summary, micropropagation of rose cultivars ranged from easy to difficult. The best source of nodal explants from greenhouse-grown plants was more than four nodes below the shoot apex. The best shoot proliferation was obtained at 2 mg/L of BA and 0.01 mg/L NAA in full-strength MS salts, while rooting of shoots improved with half-strength MS salts. The rooting of recalcitrant genotypes was improved with IAA supplementation at 1 mg/L.

References

Bressan PH, Kim Y, Hyndman SE (1982) Factors affecting *in vitro* propagation of rose. J Am Soc Hortic Sci 107: 979-990

Burger DW, Liu L, Zary KW (1990) Organogenesis and plant regeneration for immature embryos of *Rosa hybrida* L. Plant Cell Tissue Organ Cult 21: 147-152

Chu CY, Knight SI, Smith MAL (1993) Effect of liquid culture on the growth and development of miniature rose (*R. chinensis* Jacq. 'Minima'). Plant Cell Tissue Organ Cult 32: 329-334

Davies DR (1980) Rapid propagation of roses *in vitro*. Scientia Hortic 13: 285-389

Dohare SR, Shafi M, Kaicker US (1991) Micropropagation in roses. Acta Hortic 289: 107-108

Donelly DJ, Skelton FE (1989) Comparison of hydathode structure in micropropagated plantlets and greenhouse-grown 'Queen Elizabeth' rose plants. J Am Soc Hortic Sci 114: 841-846

Dorion N, Kadri M, Bigot C (1991) *In vitro* preservation at low temperature of rose plantlets usable for direct acclimatization. Acta Hortic 298: 335-339

Douglas GC, Rutledge CB, Casey AD (1989) Micropropagation of floribunda, ground cover and miniature roses. Plant Cell Tissue organ Cult 19: 55-64

Hyndman S, Hasegawa PM, Bressan RA (1982a) Stimulation of root initiation from culured rose shoots through the use of reduced concentrations of mineral salts. HortSci 17: 82-83

Hyndman S, Hasegawa PM, Bressan RA (1982b) The role of sucrose and nitrogen in adventitious root formation on cultured rose shoots. Plant Cell Tissue Organ Cult 1: 229-238

Jacobs G, Allan P, Bornman CH (1970a) Tissue culture studies on rose: Use of shoot tip explants. II. Cytokinin : gibberellin effects. Agroplantae 2: 25-28

Jacobs G, Allan P, Bornman CH (1970b) Tissue culture studies on rose: use of shoot tip explants. III. Auxin: gibberellin dffects. Agroplantae 2: 45-50

Korban M, Donnelly DJ (1994) Performance of micropropagated 'Queen Elizabeth' rose following mechanically induced stress. HortSci 29: 20-21

Krussmann G (1981) The complete book of roses. Portland, OR: Timber Press

Langford PJ, Wainwright H (1988) Influence of sucrose concentration of the photosynthetic ability of *in vitro* grown rose shoots.

Acta Hortic 227: 305-310

Lloyd D, Roberts AV, Short KC (1988) The induction in vitro of adventitious shoots in Rosa. Euphytica 37: 31-36

Metthews D, Mottley J, Horan I (1991) A protoplast to plant system in roses. Plant Cell Tissue Organ Cult 24: 173-180

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco cultures. Physiol Plant 15: 473-497

Roberts AV, Horan I, Matthews D (1990) Protoplast technology and somatic embryogenesis in Rosa. In: de Jong J (ed.) Proc Eucarpia Symp. Integration of *in vitro* techniques in ornamental plant breeding Wageningen, The Netherlands, Centre for Plant Breeding Research 110-115

Roberts AV, Walker S, Horan I (1992) The effects of growth retardants, humidity and lighting at stage III on stage IV of micropropagation in chrysanthemum and rose. Acta Hortic 319: 153-158

Rogers RB, Smith MAL (1992) Consequences of *in vitro* and vitro root initiation for minature rose production. J Hortic Sci 667: 535-540

Rout GR, Debata BK, Das P (1991) Somatic embryogenesis in callus cultures of *Rosa hybrida* L. cv. Landora. Plant Cell Tissue Organ Cult 27: 65-69

Short KC, Roberts AV (1991) *Rosa* spp. (Roses); *In vitro* culture, micropropagation, and the production of secondary products. In: Bajaj YPS (ed.) Biotechnology in agriculture and forestry. Vol. 15. Medicinal and aromatic plants III. Berlin, Germany: Spriner-Verlag: 376-397

Skirvin RM, Chu MC, Young HJ (1990) Rose. *In*: Ammirato PV, Evans DR, Sharp WR (eds.) Handbook of plant cell culture. McGraw-Hill Publishing Co., New York NY: 716-743

Tabaeezadeh A, Khosh-Kui M (1981) Anther culture of Rosa. Sci Hortic 15: 61-66