Iran Agricultural Research 15:51-67 (1996)

MICROPROPAGATION OF MINIATURE ROSE CULTIVARS

H. SALEHI AND M. KHOSH- KHUI[1]
Department of Horticulture, College of Agriculture, Shiraz University, Shiraz, Iran.
(Received: January 23, 1996)

ABSTRACT

Experiments were conducted to study the regeneration ability of 'Little Buckaroo', 'Sourati' and 'Baby Masquerade' miniature roses. Single-node explants were sterilized and cultured on establishment MS medium supplemented with various concentrations of BA in combination with different concentrations of IBA, NAA or IAA. In general, BA at 1.5 to 2.5 mg l^{-1} in combination with low concentrations of used auxins were the most suitable treatments for shoot proliferation. Subculturing was performed on the same fresh media, four times at 4-week intervals. The best treatment for rooting was quick-dip method in a sterilized 1000 mg l^{-1} NAA solution and then culturing on $^1/_2$ or full strength MS salts plus vitamins, 6 g l^{-1} agar and 30 g l^{-1} sucrose supplemented with 0.1 mg l^{-1} IAA and 0.05 mg l^{-1} NAA or IBA for 'Little Buckaroo', the same basal medium but with 0.1 mg l^{-1} IAA and 0.05 mg l^{-1} IBA for 'Baby Masquerade' and the same basal medium ($^1/_2$ MS) but with 0.1 mg l^{-1} IAA and NAA for 'Sourati'. Plantlets were transferred to the same, but growth regulator-free medium for root elongation one week after planting on rooting medium. Plants were successfully transferred to vermiculite or a mixture of 1:1:1 vermiculite, sand and leaf-mold (v/v/v). 'Little Buckaroo' acclimatized easier than the other cultivars.

---

1. Former Graduate Student (now Instructor) and Professor, respectively.

تحقیقات کشاورزی ایران

(۱۳۷۵) ۶۷-۵۱:۱۵

ریزافزایشی ارقام رزمینیاتور

حسن صالحی و مرتضی خوشخوی

به ترتیب دانشجوی سابق کارشناسی ارشد (اکنون مربی) و استاد بخش باغبانی، دانشکده کشاورزی ، دانشگاه شیراز، شیراز، ایران.

چکیده

آزمایش هایی برای مطالعه قابلیت باززایی سه رقم رز مینیاتور با نام های محلی قرمز، صورتی وهفت رنگ انجام شد. ریزنمونه های تک-گره پس از گندزدایی روی محیط استقرار موراشیگی و اسکوگ (MS) با غلظت های متفاوتی از بنزیل آدنین (BA) و غلظت های مختلف اسید ایندول بوتیریک (IBA) ، اسید نفتالن استیک (NAA) یا اسید ایندول استیک (IAA) کشت شدند. بطور معمول ،BA با غلظت ۱/۵ تا ۲/۵ میلی گرم در لیتر به همراه غلظت های کم هرکدام از اکسین های بکاررفته بهترین نتیجه را داشت. گیاهک ها، چهاربار با فاصله های چهارهفته ای، روی محیط مشابه تازه، زیرکشت شدند. بهترین تیمارهای ریشه زایی شامل فرو بری سریع در محلول گندزدائی شده ۱۰۰۰ میلی گرم در لیتر NAA و سپس کشت روی نیم غلظت یا تمام غلظت نمک های MS با ویتامین ها، ۶ گرم در لیتر آگار، ۳۰ گرم در لیتر سوکروز، ۰/۱ میلی گرم در لیتر IAA و ۰/۰۵ میلی گرم درلیتر NAA یا IBA برای رقم قرمز، محیط پایه مشابه

با ۰/۱ میلی گرم درلیتر IAA و۰/۰۵ میلی گرم در لیتر IBA برای رقم هفت رنگ، و محیط پایه مشابه (نیم غلظت نمک های MS) با ۰/۱ میلی گرم در لیتر IAA و NAA برای رقم صورتی بود. گیاهک ها یک هفته بعد از کشت روی محیط ریشه زایی به محیط های مشابه ولی بدون تنظیم کننده رشد، برای طویل شدن ریشه ها، منتقل شدند. گیاهک ها باموفقیت به ورمی کولایت یا مخلوطی با نسبت های حجمی مساوی از ورمی کولایت ، ماسه و خاکبرگ منتقل شدند. سازگارنمودن رقم قرمز آسان تر از دو رقم دیگر بود.

INTRODUCTION

Miniature roses have become increasingly popular and economically important in recent years (22, 25). *In vitro* propagation is a relatively new and promising technique for rose production (4). Rapid clonal propagation method has a great commercial value in the rose *in vitro* industry (4). Micropropagation of miniature roses also resulted in a superior and more marketable product with a higher growth rate and more compact growth than plants produced by cuttings (8).

The establishment of tissue culture propagation systems for various rose species has been described (1, 2, 3, 5, 6, 11, 12, 13, 17, 21). In this investigation, the regeneration abilities of three miniature rose cultivars, commonly cultured in Iran, were studied through micropropagation.

MATERIALS AND METHODS

Plant Materials

Three miniature roses (*Rosa chinensis* Jacq. var. minima Rehd hybrids), 'Little Buckaroo', 'Baby Masquerade' and a local cultivar, 'Sourati', were used in this study.

Establishment

Single-nodes, 1.5 to 2 cm long were prewashed in a commercial dish washer (Rika) solution (about 0.2%) for 10-15 min and then placed under running tap water until used. The explants were disinfected and/or disinfested as follows:

a. 'Little Buckaroo' and 'Sourati' were surface sterilized in 70% ethanol for 4 min and then in a 10% household bleach (containing 5.25% sodium hypochlorite) for about 20 min, followed by three rinses in sterilized distilled water.

b. 'Baby Masquerade' explants, after surface sterilization and rinsing with sterilized distilled water (as described above) were immersed in a 100 mg l^{-1} sterilized solution of gentamicin or ampicillin for 30 min and then were cultured (19).

Explants, 0.5-1.0 cm long, were used for culturing in culture vessels containing about 25 ml of Murashige and Skoog (MS) (16) medium salts and vitamins plus 30 g l^{-1} sucrose and 8 g l^{-1} agar, supplemented with 1.00, 1.25, 1.50, 1.75, 2.00, 2.25 or 2.50 mg l^{-1} benzyladenine (BA) with 0.05 mg l^{-1} indolebutyric acid (IBA), 0.10 mg l^{-1} naphthaleneacetic acid (NAA) or 0.15 mg l^{-1} indoleacetic acid (IAA) for shoot proliferation. In a separate experiment, the most suitable medium for each cultivar was supplemented with 0.1 mg l^{-1} gibberellic acid (GA_3). Cultures were kept under a 16-hr photoperiod of 1500 lux light intensity at 25±3 °C.

Subcultures

Subculturing was performed every 4 weeks to fresh, best establishment medium for each cultivar.

Rooting and Acclimatization

Media used for rooting (initiation and elongation) consisted of $^1/_4$, $^1/_2$ or full strengths of MS salts plus vitamins, 30 g l^{-1} sucrose and 6 g l^{-1} agar

supplemented with 0.1 or 0.2 mg l^{-1} IAA in combination with 0.05 or 0.1 mg l^{-1} IBA or NAA. *In vitro* derived shoots were cultured on these media after quick-dip in a sterilized rooting aqueous solution containing 1000 mg l^{-1} NAA for 5 sec. On the basis of preliminary experiments, rooting cultures were placed under 1000 lux for 2-3 days and thereafter under 1500 lux light intensity. After 7 days the shoots were transferred to the same medium without any growth regulators for root elongation. After 2 weeks, rooted plantlets were transferred to a pasteurized soil mixture in either clay or fiber pots. Soil mixtures consisted of peat moss; vermiculite; a mixture of 1:1:1 (v/v/v) vermiculite, sand and leaf-mold; a mixture of 1:2 (v/v) vermiculite and sand or a mixture of 1:1:1 (v/v/v) vermiculite, sand and loam soil. The acclimatized plants were transferred to the greenhouse after 3 weeks.

Data Recording and Analysis

The proliferation of shoots was recorded after 4 weeks. Rooting percentage as well as number and length of roots produced by each shoot in elongation media were measured after 3 weeks. All experiments were conducted as a completely randomized design with eight replications and repeated three times. Data were statistically analyzed and the means were compared using Duncan's new multiple range test (DNMRT). Data recorded as percentage were analyzed after appropriate statistical transformation.

RESULTS

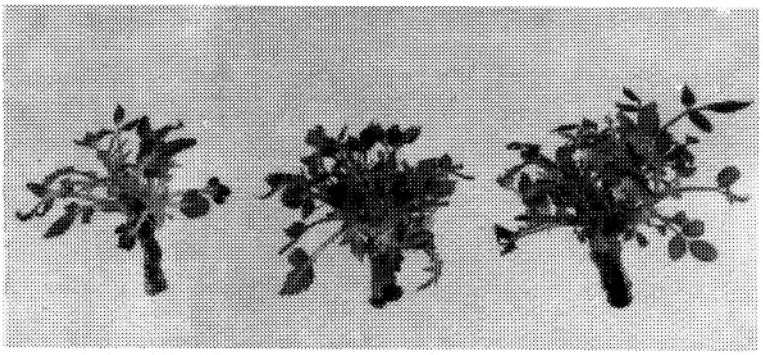
Establishment

Differences between cultivars in production of shoots in establishment media were highly significant (Table 1). On suitable medium for each cultivar, 'Little Buckaroo' had the highest and 'Baby Masquerade' had the lowest shoot proliferation (Fig. 1).

Table 1. Shoot proliferation of three miniature rose cultivars in establishment media (data are averaged over all concentrations of growth regulators).

Cultivars	Shoot number
'Little Buckaroo'	2.4 a†
'Sourati'	1.7 b
'Baby Masquerade'	1.4 c

† Means with different letters are significantly different at 1% level of probability using DNMRT.



A B C

Fig.1. Proliferation of three miniature rose cultivars on MS medium supplemented with the most suitable growth regulators for each cultivar (A= 'Baby Masquerade' 2.25 mg l^{-1} BA and 0.1 mg l^{-1} NAA, B= 'Sourati' 2.0 mg l^{-1} BA and 0.1 mg l^{-1} NAA and C= 'Little Buckaroo' 1.5 mg l^{-1} BA and 0.05 mg l^{-1} IBA).

Various auxins did not significantly affect the proliferation of cultivars, however, various concentrations of BA showed significant

differences (Table 2). Combinations of 1.25-2.50 mg l^{-1} BA with one of the used auxin sources for 'Little Buckaroo', 1.75-2.00 mg l^{-1} BA with 0.1 mg l^{-1} NAA for 'Sourati' and 2.25 mg l^{-1} BA with 0.1 mg l^{-1} NAA for 'Baby Masquerade' were the most suitable treatments. Use of 0.1 mg l^{-1} GA_3 in the media resulted in the production of longer shoots, but did not significantly affect the proliferation (data not shown).

Shoot forming capacity increased on cultures not subcultured after 4 weeks. However, some shoots showed gradual chlorosis and finally dried out. Nevertheless, some explants survived on establishment medium for 3 months or longer, and produced a considerable number of shoots and roots (Fig. 2).

Subcultures

Established shoots of different cultivars that were subcultured, differed significantly for proliferation in all multiplication stages except for establishment and the fourth subculture (Fig. 3). The proliferation in establishment stage for 'Little Buckaroo' was similar to the second and third subcultures. Generally, 'Sourati' and 'Baby Masquerade' proliferations were lower than 'Little Buckaroo'(Fig. 3). 'Sourati' had the highest proliferation in establishment and first subculture stages while 'Baby Masquerade' had the highest proliferation at establishment, first and second subcultures (Fig. 3). Among the multiplication stages, the first subculture resulted in the highest proliferation and after third subculture, proliferation was negligible (Table 3).

Rooting and Acclimatization

'Little Buckaroo' had the highest rooting percentage and largest mean root length per plantlet, while 'Sourati' had the highest number of roots per plantlet (Table 4). For all cultivars, full strength MS salts resulted in the

highest rooting percentage, root number and mean root length per plantlet, and $^1/_4$ MS was completely ineffective (Table 4).

Table 2. Number of shoots produced by single-node explants of three miniature roses in establishment media supplemented with various growth regulators.

BA (mg l^{-1})	'Little Buckaroo'	'Sourati'	'Baby Masquerade'
		IAA (0.15 mg l^{-1})	
1.00	1.7 bA†	1.0 bA	1.2 aA
1.25	2.0 abA	1.0 bB	1.0 aAB
1.50	2.5 abA	1.5 abB	1.0 aB
1.75	2.7 abA	2.2 aA	1.7 aA
2.00	3.0 aA	1.5 abB	1.5 aB
2.25	2.7 abA	2.0 abAB	1.5 aB
2.50	2.0 abA	1.7 abA	1.0 aA
		IBA (0.05 mg l^{-1})	
1.00	1.0 bA	1.5 aA	1.0 aA
1.25	2.5 aA	2.2 aA	1.2 aB
1.50	3.2 aA	2.2 aB	1.5 aB
1.75	2.2 aA	2.0 aA	1.0 aB
2.00	2.7 aA	1.5 aB	1.0 aB
2.25	2.7 aA	1.7 aB	1.2 aB
2.50	2.7 aA	1.5 aB	1.2 aB
		NAA (0.10 mg l^{-1})	
1.00	1.0 bA	1.0 bA	1.5 abA
1.25	2.2 aA	1.0 bB	2.0 abA
1.50	2.7 aA	1.7 abB	1.5 abB
1.75	2.7 aA	2.5 aA	1.0 bB
2.00	3.0 aA	2.7 aA	1.7 abB
2.25	2.7 aA	2.0 abA	2.5 aA
2.50	2.2 aA	2.0 abA	1.2 bA

† Means followed by the same letters (capital letters for rows and small letters for columns) are not significantly different at 5% level of probability using DNMRT.

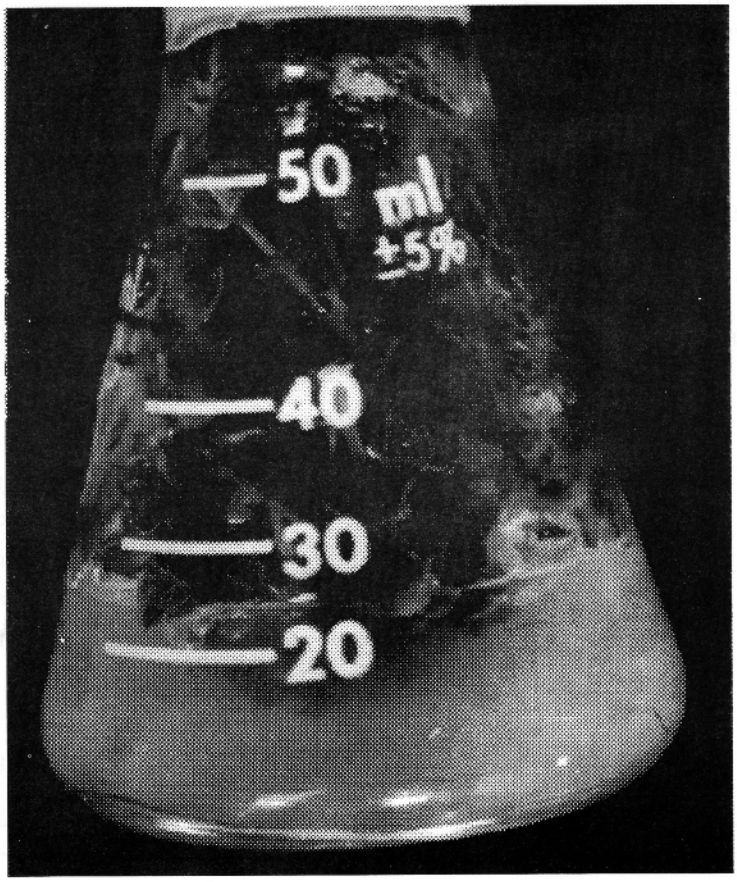


Fig. 2. Proliferation and rooting of 'Little Buckaroo' single-node explants cultured on a MS + 2.0 mg l^{-1} BA and 0.1 mg l^{-1} NAA after three months.

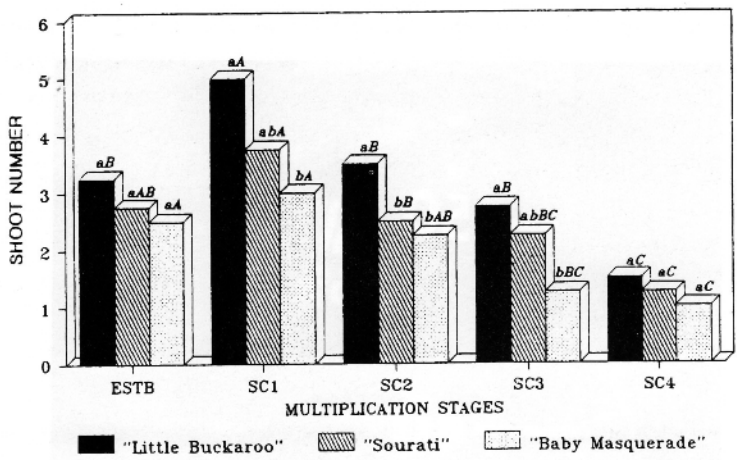


Fig. 3. Proliferation of different cultivars in various multiplication stages (ESTB=establishment, SC1= first subculture, SC2= second subculture, SC3= third subculture and SC4= fourth subculture). Bars with the same letters (small letters between cultivars and capital letters within each cultivar) are not significantly different at 5% level of probability using DNMRT.

Table 3. Proliferation at different multiplication stages (data are averaged over the cultivars).

Stage	Shoot number (mean)
Establishment	2.8 b†
First subculture	3.9 a
Second subculture	2.7 bc
Third subculture	2.1 c
Fourth subculture	1.2 d

† Means followed by the same lettres are not significantly different at 5% level of probability using DNMRT.

'Little Buckaroo' had the highest rooting percentage when cultured on full strength MS medium supplemented with combinations of 0.1 mg l^{-1} IAA and 0.05 mg l^{-1} NAA or IBA (Table 5). In 'Sourati', the highest rooting percentage and root number per plantlet were observed on full or $^1/_2$ strength MS salt media supplemented with combinations of 0.1 mg l^{-1} IAA and NAA (Table 5). 'Baby Masquerade' had the highest rooting percentage and root number per plantlet with full or $^1/_2$ strength MS salt media supplemented with combinations of 0.1 mg l^{-1} IAA and 0.05 mg l^{-1} IBA (Table 5).

Plantlets were successfully transferred to vermiculite or a mixture of 1:1:1 vermiculite, sand and leaf-mold (v/v/v). 'Little Buckaroo' acclimatized easier than the other cultivars.

Table 4. Rooting of different cultivars (data are averaged over the growth regulators and MS salt strengths) and various MS salt strengths (data are averaged over three cultivars and the growth regulators) in relation to rooting variables.

Variables	Rooting percentage	Root number plantlet^{-1}	Mean root length plantlet^{-1} (mm)
Cultivars:			
'Little Buckaroo'	47.6 a†	1.3 b	0.6 a
'Sourati'	45.1 b	1.6 a	0.4 b
'Baby Masquerade'	23.4 c	0.8 c	0.4 b
MS salt strengths:			
Full	63.3 a	1.9 a	0.8 a
$^1/_2$	47.7 b	1.5 b	0.6 b
$^1/_4$	5.1 c	0.2 c	0.1 c

† Means followed by the same letters in each column are not significantly different at 1% level of probability using DNMRT.

Table 5. Comparisons between different rooting variables in three cultivars, three weeks after culture on rooting media with various MS salt strengths and growth regulators [growth regulators (in mg l^{-1}): 1= IAA 0.1+ IBA 0.05, 2= IAA 0.1+ NAA 0.05, 3= IAA 0.1+ IBA 0.1, 4= IAA 0.1+ NAA 0.1, 5= IAA 0.2+ IBA 0.05, 6= IAA 0.2+ NAA 0.05, 7= IAA 0.2+ IBA 0.1 and 8= IAA 0.2+ NAA 0.1].

Cultivar	Growth regulator treatments	MS salt strengths								
		full	1/2	1/4	full	1/2	1/4	full	1/2	1/4
		Rooting percentage			Root number plantlet^{-1}			Root length plantlet^{-1} (mm)		
'Little Buckaroo'	1	100.00aA†	51.67eB	20.00aC	3.00bA	1.33bB	1.00aB	1.27aA	1.03aA	0.57aB
	2	100.00aA	66.67cdB	21.67aC	6.33aA	1.67abB	1.00aB	1.07abA	1.03aA	0.73aA
	3	86.67bA	71.67bcB	0.00bC	1.67cA	1.67abA	0.00bB	0.83abA	0.63aA	0.00bB
	4	67.33cA	72.67bcA	0.00bB	1.67cA	1.00bA	0.00bB	0.87abA	0.93aA	0.00bB
	5	65.00cdA	68.33bcdA	0.00bB	1.00cdA	1.00bA	0.00bB	0.80abA	0.93aA	0.00bB
	6	68.67cA	76.67bA	0.00bB	1.00cdA	1.00bA	0.00bB	0.73bA	0.83aA	0.00bB
	7	55.00dB	90.00aA	0.00bC	1.67cB	2.67aA	0.00bC	1.07abA	0.73aA	0.00bB
	8	0.00cB	60.00deA	0.00bB	0.00dB	2.00abA	0.00bB	0.00cB	1.03aA	0.00bB

Table 5., (continued)

	1	53.33cB	78.33bA	26.67bC	1.00cB	2.33bA	1.33aB	0.87abA	0.53aA	0.67aA
	2	53.00cB	88.33bA	45.00aB	1.67bcB	5.00aA	1.33aB	0.53bA	0.46aA	0.43abA
	3	75.00bA	68.33cA	0.00dB	1.67bcB	2.67bA	0.00bC	0.50bA	0.50aA	0.00bB
'Sourati'	4	91.67aA	91.67aA	10.00cB	5.00aA	4.67aA	1.00abB	0.27cB	0.53aA	0.73aA
	5	41.67dA	38.33eA	0.00dB	1.33cA	1.00cA	0.00bB	0.83abA	0.70aA	0.00bB
	6	45.00cdB	66.67cA	0.00dC	1.33cA	1.00cA	0.00bB	1.00aA	0.70aA	0.00bB
	7	76.67bA	51.67dB	0.00dC	1.67bcA	1.00cA	0.00bB	0.70abA	0.40aA	0.00bB
	8	86.67abA	0.00fB	0.00dB	2.67bA	0.00cB	0.00bB	0.50bA	0.00bB	0.00bB
	1	93.33aA	91.67aA	0.00aB	4.00aA	4.33aA	0.00aB	1.33aB	2.23aA	0.00aC
	2	85.00bA	16.67bB	0.00aC	2.33bA	1.33bB	0.00aC	1.47aA	1.00bB	0.00aC
	3	61.67dA	0.00cB	0.00aB	1.33bA	0.00cB	0.00aB	0.77bcA	0.00cB	0.00aB
'Baby Masquerade'	4	73.33cA	0.00cB	0.00aB	1.67bA	0.00cB	0.00aB	0.87bA	0.00cB	0.00aB
	5	50.00eA	0.00cB	0.00aB	1.33bA	0.00cB	0.00aB	1.00bA	0.00cB	0.00aB
	6	40.00eA	0.00cB	0.00aB	1.33bA	0.00cB	0.00aB	0.90bA	0.00cB	0.00aB
	7	0.00fA	0.00cA	0.00aA	0.00cA	0.00cA	0.00aA	0.00dA	0.00cA	0.00aA
	8	50.00eA	0.00cB	0.00aB	2.33bA	0.00cB	0.00aB	0.47cA	0.00cB	0.00aB

† Means followed by the same letters (capital letters for rows and small letters for columns) are not significantly different at 1% level of probability, using DNMRT.

DISCUSSION

BA at concentrations of 1.5-2.5 mg l^{-1} in combination with low rates of auxins was the most suitable treatments for *in vitro* multiplication of the three miniature rose cultivars studied. This is in accordance with the results obtained by several other investigators (7, 9, 12, 20) who worked on various rose species. 'Little Buckaroo' required lower levels of BA than 'Baby Masquerade', while 'Sourati' was intermediate. Probably, genotypic variation accounts for these differences (2, 12).

In the present investigation, GA_3 treatment induced longer shoots but did not affect proliferation. Contradictory results were reported for improvement (23) and reduction (10) of shoot proliferation of roses in media supplemented with GA_3. This contradiction may be due to different amounts of hormones needed for various rose genotypes.

Production of considerable shoots and roots in a few cultures after 2 to 3 months needs further investigation to find the optimum conditions for both shoot and root production at the multiplication stage.

In this study, the general reduction in proliferation after first subculture might be due to an altered endogenous cytokinin level in plant tissue after continuous subcultures (15). In contrast to this result, Campos and Pais (5) reported the highest shoot proliferation of 'Rosamini' at third subculture and Chu *et al.* (6) showed the same proliferation in all four subcultures. Further experiments are required to determine the optimum medium for each subculture.

In the present study, using a quick-dip method of auxin before culturing the shoots on rooting media with low concentrations of auxin was successful. This method may be useful for *in vitro* rooting of plants that are sensitive to high auxin concentrations in rooting medium. Combinations of low concentrations of different auxins were the best treatments for rooting of

three miniature rose cultivars. Similar findings were reported by Khosh-Khui and Sink (13) and Rahman *et al.* (17).

Both full and $^1/_2$ MS salts strengths were suitable for rooting media in all cultivars used in this experiment. However, 'Little Buckaroo' had better rooting on full than $^1/_2$ MS medium. Effectiveness of the full MS (14, 15, 18) or reduced MS salt strengths media (5, 20, 24) for best *in vitro* rooting of roses have been reported.

When shoots were kept in the rooting medium for more than 10 days, root-tips became brown in color and plantlets died after a few days. The problem was solved by transferring the plantlets from rooting medium to root elongation medium described for 'Rosamini' (5). It may be concluded that auxins are necessary just for root initiation, but not for subsequent root development of miniature roses.

ACKNOWLEDGEMENT

Constructive suggestions of Dr. M. Niknejad (deceased), Dr. A. Hassan-Pour, Dr. Y. Emam and Dr. D.P. de Vries (from Agricultural Research Department, Wageningen, the Netherlands) are appreciated.

LITERATURE CITED

1. Alderson, P.G., J. McKinless and R. D. Rice. 1988. Rooting of cultured rose shoots. Acta Hortic. 226:175-182.
2. Arnold, N.P., M.R. Binns, N.N. Barthakur and D.C. Cloutier. 1992. A study of the effect of growth regulators and time of plantlet harvest on the *in vitro* multiplication rate of hardy and hybrid tea roses. J. Hortic. Sci. 67:727-735.
3. Bhat, M.S. 1992. Micropropagation in rose. Indian Hortic. 37:17-19.

4. Bressan, P.H., Y.J. Kim, S.E. Hyndman, P.M. Hasegawa and R.A. Bressan. 1982. Factors affecting *in vitro* propagation of rose. J. Amer. Soc. Hortic. Sci. 107:979-990.
5. Campos, P.S. and M.S.S. Pais. 1990. Mass propagation of the dwarf rose cultivar 'Rosamini'. Scientia Hortic. 43:321-330.
6. Chu, C.Y., S.L. Knight and M.A.L. Smith. 1993. Effect of liquid culture on the growth and development of miniature rose (*Rosa chinensis* Jacq. 'Minima'). Plant Cell Tis. Org. Cult. 32:329-334.
7. Davies, D.R. 1980. Rapid propagation of roses *in vitro*. Scientia Hortic. 13:385-389.
8. Dubois, L.A.M., J. Roggemans, G. Soyeurt and D.P. de Vries. 1988. Comparison of the growth and development of dwarf rose cultivars propagated *in vitro* and *in vivo* by softwood cuttings. Scientia Hortic. 35:293-299.
9. Hasegawa, P.M. 1979. *In vitro* propagation of rose. HortScience 14:610-612.
10. Hasegawa, P.M. 1980. Factors affecting shoot and root initiation from cultured rose shoot tips. J. Amer. Soc. Hortic. Sci. 105:216-220.
11. Horn, W.A.H. 1992. Micropropagation of Rose. In: Y.P.S. Bajaj (ed.). Biotechnology in Agriculture and Forestry. Springer-Verlag, Germany. Vol. 20, 320-342.
12. Khosh-Khui, M. and K.C. Sink. 1982a. Micropropagation of new and old world rose species. J. Hortic. Sci. 57:315-319.
13. Khosh-Khui, M. and K.C. Sink.1982b. Rooting-enhancement of *Rosa hybrida* for tissue culture propagation. Scientia Hortic. 17:371-376.
14. Korban, M. and D.J. Donnelly. 1994. Performance of micropropagated 'Queen Elizabeth' rose following mechanically induced stress. HortScience 29:20-21.

15. Mederos, S. and M.J. Rodriguez Enriquez. 1987. *In vitro* propagation of 'Golden Times' roses. Factors affecting shoot tip and axillary bud growth and morphogenesis. Acta Hortic. 212:619-624.
16. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.
17. Rahman, S.M., M. Hossain, A.K.M. Rafiul Islam and O.I. Joarder. 1992. Effects of media composition and culture conditions on *in vitro* rooting of rose. Scientia Hortic. 52:163-169.
18. Roberts, A.V., D. Lloyd and K.C. Short. 1990. *In vitro* procedures for the induction of tetraploidy in a diploid rose. Euphytica 49:33-38.
19. Salehi, H. 1995. *In vitro* propagation of miniature rose cultivars. M.S. Thesis, Shiraz Univ., Shiraz, Iran.
20. Skirvin, R.M. and M.C. Chu. 1979. *In vitro* propagation of 'Forever Yours' rose. HortScience 14:608-610.
21. Skirvin, R.M., M.C. Chu and H.J. Young. 1990. Rose. In: P.V. Ammirato, W.R. Sharp, D.A. Evans and Y.P.S Bajaj (eds.). Handbook of Plant Cell Culture. McGraw-Hill Pub. Inc., New York, U.S.A. Vol. 5 (Ornamental Species), 716-743.
22. Tjosvold, S.A., M.J. Wu and M.S. Reid. 1994. Reduction of postproduction quality loss in potted miniature roses. HortScience 29:293-294.
23. Valles, M. and P. Boxus. 1987. Micropropagation of several *Rosa hybrida* L. cultivars. Acta Hortic. 212:611-617.
24. Walter, F. and A. Sauer. 1986. *In vitro* mutagenesis in roses. Acta Hortic. 189:37-46.
25. Zieslin, N. and M.J. Tsujita. 1990. Response of miniature roses to supplementary illumination. I. Light intensity. Scientia Hortic. 42:113-121.