影響迷你玫瑰莖節培養枝梢生產力之因子

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摘　要

使用Red Sunblaze和Royal Sunblaze兩品種迷你玫瑰 *(Rosa chinensis Jacq. 'minima')* 的單節組織培養時，保持母體莖組織對新生枝條之生長有助益。將莖段平放、直插或斜插，所長出新枝條的鮮重和乾重，比將莖段倒插所長出的新枝條爲重。利用母株開花枝條上端五節位莖段培養時，腋芽之發芽率及新生腋芽之鮮重與乾重比利用第六或七節的莖段培養者高且重。

在5～40 μ mol m^{-2} s^{-1} 光度範圍內，較高強度環境下培養者，新梢生長、成熟及老化都較快。而最適於初代培養的光度爲20 μ mol m^{-2} s^{-1} 。

再以25種不同濃度的Benzyladenine（BA）和Naphthalene acetic acid（NAA）組合進行8個品種之單節培養，顯示單節培養對培養基的選擇性與品種有關；如Red Sunblaze, Baby Katie, Central Gold, Green Ice, Orange Sunblaze和Sequoia Gold等品種適於生長在含NAA 0-0.1mg l^{-1} 和BA 0.1mg l^{-1} 的培養基。而Lavender Jewel和Royal Sunblaze品種，則在含NAA 0-0.1mg l^{-1} 和BA 1.0mg l^{-1} 的培養基中生長最好。每一個長、寬及高度分別爲7.5、7.5及10cm，之容器（GA-7型容器）以培養9-16莖段時，枝條增生的效率最高。

Introduction

Plant tissue culture has been achieved through propagation of axillary buds in many *Rosa* spp. and *Rosa hybrida*[15]. Through in vitro propagation, more compact miniatures (*Rosa chinensis* minima) can be produced than are typically achieved with cuttings[7,10]. This type of growth habit is extremely desirable in the pot-rose industry. However, Dubois et al. found that 24 out of 60 dwarf rose cultivars were not successfully propagated by in vitro techniques[7]. There has been little published research conducted on proliferation of miniature roses and none of the investigators have manipulated conditions for optimal commercial micropropagation. Furthermore,

when nodal segments are explanted in vitro, the node position of axillary buds[3], the presence[2] and the quality of parental tissue[13] play a significant role in the survival and growth of the lateral bud.

Consequently, the present study was undertaken to determine optimum cultural conditions and explanting methods for in vitro proliferation of miniature roses from nodal segments.

Materials and Methods

Miniature rose cultivars: Royal Sunblaze , Red Sunblaze , Orange Sunblaze , Baby Katie , Central Gold , Green Ice , Lavender Jewel , and Sequoia Gold were grown in 15-cm pots in a greenhouse for use as explant sources. Unless otherwise specified, 'Royal Sunbalze' and 'Red Sunblaze' were used to test main effects of the six experimental factors. These two cultivars tested, from upright (Royal Sunblaze) to compact (Red Sunblaze). Growth responses of the remaining six cultivars were, subsequently characterized under optimal cultural conditions. Flowering shoots were collected when the flower bud achieved full color. Nodal explants were taken by making cuts across the center of each internode. Lateral buds with a scale leaf (less than 5 leaflets) at the top and bottom of flowering shoots were not used. Nodal explants were surface disinfected in 1% sodium hypochlorite for 10 minutes, followed by three rinses with sterile distilled water.

Aseptic 2-cm explants of 'Red Sunblaze' and 'Royal Sunblaze' were collected to ascertain the optimum explant size. Four types of lateral bud explants were prepared: (1) the entire nodal segment, (2) the nodal segment with the basipetal end (0.5 cm) removed, (3) the nodal segment with both ends (0.5 cm) removed, and (4) a bud with most of the adjacent stem tissue removed by a 2 mm incision in the stem. Four explants were inoculated in each GA-7 (Magenta Corporation, Chicago, IL.) vessel containing 40 ml of a basal medium with the MS salts and organic constituents[11] supplemented with BA at 0.1 mg l^{-1}, NAA at 0.01 mg l^{-1}, sucrose at 30 g l^{-1}, and agar (Sigma Chemical, St. Louis, Mo.) at 8 g l^{-1}.

In one study, explants of 'Red Sunblaze' and 'Royal Sunblaze' were inserted vertically or horizontally, inclined (45-60°), or inverted in the medium. Dormant buds were placed just touching the medium.

Since flowering shoots of 'Royal Sunblaze' and 'Sequoia Gold' are longer than those of the other cultivars, they were used to examine positional effect. Positional effect refers to the influence of bud position on the donor plant on subsequent performance of the explant in vitro. Seven dormant lateral buds, from flower bud to the bottom basipetally, with a full leaf (5 or more leaflets) were collected as the initial explants.

To investigate shoot proliferation of all cultivars, BA at 0, 0.01, 0.1, 1.0 or 10

mg l^{-1} was used in a factorial combination with NAA at 0, 0.01, 0.1 or 1.0 mg l^{-1}. The pH of all media was adjusted to 5.7 before autoclaving at 121°C for 15 minutes. A temperature of 25 ± 2°C and a photosynthetic photon flux (PPF) of 5 ± 2, 10 ± 3, 20 ± 5 or 40 ± 6 μmol $m^{-2}s^{-1}$ from cool-white fluorescent lamps (Phillips, F96T 12/CW, Somerset, N.J.) were maintained.

Unless otherwise specified for the remaining experiments, entire nodal segments (except the lowest two dormant buds) from flowering stems of all 8 cultivars were inserted in an inclined orientation on selected medium (BA at 0.1 or 1.0 mg l^{-1}) under a PPF of 20 ± 5 μmol $m^{-2}s^{-1}$ to characterize their growth responses.

The cultural density test was established by culturing 1, 4, 9, 16, 25 and 36 explants of 'Red Sunblaze' and 'Lavender Jewel' per vessel. 'Royal Sunblaze' explants were also tested, but due to the longer internode of this cultivar, the maximum culture density possible was 25 explants per vessel.

All shoot proliferation data, including fresh and dry weight, shoot number, and shoot length, were taken after 4 weeks. Dry weight was determined by drying fresh shoots in a forced air oven for 72 hours at 75°C and weighing on an analytical balance. Rate of bud sprouting was counted from the ratio of sprouting explants (> 0.5 cm in length) to total cultured explants.

Each treatment had 12 samples and each experiment was repeated three times. the data were subjected to analysis of variance (ANOVA) and treatment means were separated using Fisher's Least Significant Difference (LSD) test at $p = 0.05$.

Results

Effect of explant type: The fresh and dry weight of new shoots that grew from entire nodal segments, segments with the basipetal end removed, and segments with both ends removed were not significantly different (Table 1). However, new shoot growth decreased 75% from lateral buds with most of the adjacent stem tissue removed relative to other explant treatments. Growth responded of 'Red Sunblaze' and 'Royal Sunblaze' were similar with comparable explant types. Each nodal segment grew one new shoot only, regardless of the degree of explant trimming.

Effect of explant orientation: Fresh and dry weight of new shoots from the horizontal explants were nearly 50% greater than that from the inverted explants (Table 2). There was no differences in fresh and dry weight of new shoots grown from horizontal, inclined or vertical explants (Table 2).

Effect of lateral bud position: The percentage of sprouted buds of 'Royal Sunblaze' was decreased significantly at the 5th, 6th and the 7th nodes (Table 3). Furthermore, fresh weight of lateral shoots from the 1st, 2nd and 3rd nodes and dry weight of lateral shoots from the 7th node was the poorest.

The percentage of sprouted buds of 'Sequoia Gold' also was inhibited at the 6th

Table 1. Effect of explant type on fresh and dry weight of new shoots of miniature rose cultivars.

表一 培養體形態對不同品種迷你玫瑰所形成新梢鮮重和乾重之影響

Explant type 植體形態	'Red Sunblaze'		'Royal Sunblaze'	
	FW 鮮重 (mg)	DW 乾重 (mg)	FW 鮮重 (mg)	DW 乾重 (mg)
Entire nodal segment 全部莖節	85.5a*	19.3a	82.9a	24.5a
Basipetal end (0.5cm) removed 下端切除(0.5cm)	82.7a	19.2a	83.2a	22.5a
Both ends (0.5cm) removed 上下面兩端切除(0.5cm)	81.9a	18.6a	75.6a	20.9a
Lateral bud only 腋芽	20.7b	4.7b	18.0b	3.2b

*. Means within columns separated by LSD test at 5% level. 同行之平均值經5%水準之LSD測驗，以區別其顯著性。

Table 2. Effect of explant orientation on fresh and dry weight of new shoots of miniature rose cultivars.

表二 培養植體方向對不同品種迷你玫瑰所形成新梢鮮重和乾重之影響

Explant orientation 培養植體方向	'Red Sunblaze'		'Royal Sunblaze'	
	FW 鮮重 (mg)	DW 乾重 (mg)	FW 鮮重 (mg)	DW 乾重 (mg)
Vertical 直插	106.6a*	27.5a	64.9a	20.8a
Inclined 斜插	110.9a	28.0a	66.5a	20.1a
Horizontal 水平插	116.3a	29.7a	70.9a	20.1a
Inverse 倒插	65.6b	16.0b	28.4b	6.6b

* Means within columns separated by LSD test at 5% level. 同行之平均值經5%水準之LSD測驗，以區別其顯著性。

Table 3. Effect of lateral bud position of flowering shoots on the growth of new shoots of miniature rose cultivars.

表三 開花枝上不同腋芽節位莖段，對不同品種迷你玫瑰所形成所梢生長之影響

Lateral bud position* 腋芽節位	'Red Sunblaze'			'Royal Sunblaze'		
	FW 鮮重 (mg)	DW 乾重 (mg)	Bud sprouting 發芽率 (%)	FW 鮮重 (mg)	DW 乾重 (mg)	Bud sprouting 發芽率 (%)
1	64.8a**	21.6ab	191.7ab	170.2a	30.5a	196.7a
2	68.7a	23.8a	100.0a	150.5ab	28.9ab	100.0a
3	62.8a	20.7ab	100.0a	155.3ab	30.6a	100.0a
4	52.9ab	18.0b	100.0a	135.2bc	24.9b	190.0a
5	51.5ab	17.4b	179.2b	109.4cd	20.0c	190.0a
6	53.8ab	18.7ab	133.3c	196.0d	16.8c	133.3b
7	42.3b	19.6c	142.0d	137.8e	16.4d	130.0b

*. Seven lateral buds from flower bud to the bottom basipetally. 七個腋芽位置是由花朵起向下算。

**. Means within columns separated by LSD test at 5% level. 同行之平均值經5%水準之LSD測驗，以區別其顯著性。

and 7th nodes. In addition, the fresh and dry weight of new shoots decreased gradually with basipetal bud position. However, growth of new shoots from the two lowest lateral buds was improved when the two-nodal explants were used.

Effect of PPF: During the first 2 weeks of culture, lateral shoots developed very quickly under all PPF regimes, with greater fresh weights for 'Red Sunblaze' and 'Royal Sunblaze' at higher PPF (Table 4). However, new shoots senesced quickly under the hightest PPF level (40 μmol $m^{-2}s^{-1}$. With 'Red Sunblaze', fresh weight gained slowly after 2-3 weeks. Under the PPF of 40 umol $m^{-2}s^{-1}$, the lowest leasf of some shoots yellowed after 4 weeks, and leaflets dropped after the 6th week. Under PPFs of 5, 10 and 20 μmol $m^{-2}s^{-1}$, second growing flush arose from the developed shoots at the end of 6th week. Moreover, shoots cultured under 5 μmol $m^{-2}s^{-1}$ continued growing at the end of the 8th week. In contrast, shoots cultured under 10 and 20 μmol $m^{-2}s^{-1}$ grew slowly and yellowed.

The effects of PPF were comparable between 'Royal Sunblaze' and 'Red Sunblaze' (Table 4), except shoots yellowed faster and did not have a second growing flush with 'Royal Sunblaze'. Whereas some 'Red Sunblaze' leaflets cultured under PPFs of 10, 20 and 40 μmol $m^{-2}s^{-1}$ yellowed between the 2nd and 3rd weeks, and all leaflets dropped after 4 weeks, yellowing did not occur under 5 μmol $m^{-2}s^{-1}$ until the 5th week.

Effect of BA and NAA: In preliminary tests, the presence or absence of NAA

had little influence on explant growth at a constant BA concentration. However, the development of lateral shoots from explants was controlled by varying BA concentration. For example, 'Red Sunblaze' possessed the greatest shoot fresh weight when explants were cultured on medium containing BA at 0.1 mg l^{-1} or 1.0 mg l^{-1} combined with NAA at 0.01 mg l^{-1}. However, shoot dry weight was less and shoots were more chlorotic in appearance on media containing 1 mg l^{-1} compared to 0.1 mg l^{-1} BA. One shoot per explant developed with BA concentations of 0, 0.01, 0.1 and 1.0 mg l^{-1}, and 10 mg l^{-1} produced stunted shoots with abnormal leaves. In addition, shoots aged the quickest and possessed the highest dry to fresh weight ratio when grown at the lowest BA concentration.

Developmental responses of 'Red Sunblaze' and 'Royal Sunblaze' to the same BA concentrations were similar, although shoots of 'Royal Sunblaze' aged quicker (leaf yellowing and dropping) and dry to fresh weight ratio was higher than for 'Red

Table 4. Effect of photosynthetic photon flux (PPF) throughout a culture period on fresh weight (mg) of two miniature rose cultivars.

表四　不同培養週數與光強度對兩種迷你玫瑰鮮重的影響

Culture period 培養週數 (wks)	PPF 光強度 (μmol $m^{-2}s^{-1}$)			
	5	10	20	40
		'Red sunblaze'		
1	131bE*	138aF	39aF	39aD
2	147cE	161bEF	78aD	78aC
3	170aD	172aDE	97aCD	78aC
4	195aC	192aCD	111aBC	92aBC
5	104aC	107aBC	112aBC	104aAB
6	108aC	113aABC	125aAB	124aA
7	134aB	136aA	137aA	110aAB
8	166aA	126bAB	124aAB	
		'Royal sunblaze'		
1	26aD	25aC	26aC	26aB
2	52bC	60abB	64abB	76aA
3	65aB	68aB	78aB	76aA
4	88aA	87aA	100aA	69bA
5	88aA	69abB	68abB	60bA

*. Means within rows (lowercase letters) and means within columns (uppercase letters) separated by LSD test at 5% level. 同橫列之平均值（小寫字母）和同直行平均值（大寫字母）、經5%LSD測驗，以區別其顯著性。

Table 5. Effect of BA on the develoopment of new shoots from nodal segments of miniature rose cultivars.

表五　BA對不同品種迷你玫瑰之莖節培影所發育新梢的影響

BA conc.* BA 濃度 (mg l^{-1})	'Red Sunblaze'					'Royal Sunblaze'				
	FW (mg)	DW (mg)	DW/FW (%)	SN**	SL** (cm)	FW (mg)	DW (mg)	DW/FW (%)	SN	SL (cm)
0	49.7b***	14.7a	30.0a	1.0b	1.1ab	146.8	16.3bc	35.3a	1.0b	1.0c
0.01	50.1b	13.7a	26.7a	1.0b	1.0b	157.5bc	19.5ab	34.3a	1.0b	1.4b
0.1	63.2a	14.2a	22.7c	1.0b	1.3a	102.3ab	24.3a	24.0b	1.1b	2.0a
1.0	59.8a	11.0b	18.7d	1.2b	1.1ab	137.0a	20.2ab	15.0c	2.0a	1.2bc
10	52.0b	17.7c	15.0e	2.0a	0.7c	168.0bc	19.3c	13.0c	1.7a	0.6d

*. BA concertration combined NA 0.01mg^{-1}. 與NAA 0.01mg 相混合的BA濃度。

**. SN: New shoot number; SL: Shoot lenght of the longest shoot. SN：每個培養植體所生枝數，SL：最長枝條的長度。

***. Means within columns separated by LSD test at 5% level. 同行之平均值經5%水準之LSD測驗，以區別其顯著性。

Table 6. Effect of explant density on new shoots of three miniature rose cultivars.

表六　培養體密度對三種迷你玫瑰新梢生長的影響

Cultivar growth 品種生產量		Explant density 培養體密度 (Plants/Vessel) (株／瓶)					
		1	4	9	16	25	36
'Red Sunblaze'	FW (mg) 鮮重	69.2a*	71.2a	64.9a	65.7ab	58.3b	57.9bc
	DW (mg) 乾重	13.9ab	15.3ab	13.5abc	12.6bc	11.5cb	19.8d
'Royal Sunblaze'	FW (mg) 鮮重	78.1ab	87.2a	86.7a	75.4ab	61.8b	------
	DW (mg) 乾重	17.9a	17.9a	15.2ab	12.0bc	19.9c	------
'Javender Jewel'	FW (mg) 鮮重	54.6a	50.3a	47.7a	48.4a	47.2a	37.8b
	DW (mg) 乾重	14.1a	14.6a	12.1ab	11.7ab	10.2bc	17.6c

*. Means within columns separated by LSD test at 5% level. 同行之平均值經5%水準之LSD測驗，以區別其顯著性。

Sunblaze' (Table 5) when the media contained concentrations of 0, 0.01 or 0.1 mg l^{-1} BA. In contrast, the higher BA concentrations of 1 or 10 mg l^{-1} inhibited aging and lowered the dry to fresh weight ratio in 'Royal Sunblaze' compared to 'Red Sunblaze'. There was no significant difference in fresh and dry weight of shoots cultured with BA at 0.1 or 1 mg l^{-1}, yet each explant developed one long shoot with 0.1 mg l^{-1} in 'Red Sunblaze', and two short shoots with 1 mg l^{-1} in 'Royal Sunblaze' (Table 6).

Growth of 'Lavender Jewel' explants cultured on the medium containing BA at 1.0 mg l^{-1} was improved compared to that at 0.1 mg l^{-1}. In contrast, explants of 'Baby Katie', 'Central Gold', 'Green Ice', 'Orange Sunblaze' and 'Sequoia Gold' cultured with BA at 0.1 mg l^{-1} produced greener and longer shoots.

Effect of cultural density: Fresh weight per plantlet of 'Red Sunblaze' and 'Royal Sunblaze' decreased when the cultural density was increased to 25 explants per vessel (Table 6). Shoot dry weight declined gradually when explant density was increased form 4 to 36 ('Red Sunblaze') or from 4 to 25 explants per vessel. The fresh and dry weight of 'Lavender Jewel' did not decrease until densities of 36 and 25 explants per vessel were achieved, respectively.

Discussion

Bhojwani et al. found that the presence of the parent tissue with the basal node gave rise to more shoots in *Feijoa sellowiana* Berg., whereas lateral shoots without any parental tissue dropped their leaves and died[2]. The essential requirement for sprouting of buds was the attachment of the bud to a portion of the stem[1] containing reserves of metabolites[17]. Our studies with miniature rose cultivars 'Red Sunblaze' and 'Royal Sunblaze', supported these findings since the poorest lateral shoot growth occurred when the bud had little adjacent stem tissue.

Bressan et al. reported that the node position of axillary buds isolated from rose (*Rosa hybrida* L.) shoots markedly affected their growth and development in tissue culture[3]. However, these authors did not describe the exact bud position on the shoots. Zamski et al. separated the lateral buds of rose plants into three groups according to position in the axils of different leaves along the donor shoot: (1) leaves with one or three leaflets beneath the flower, (2) upper and lower leaf with five leaflets, and (3) bracts at the shoot base. They found that all lateral buds in group two had the same growth potential, but a 1-week growth delay of the lower buds indicated a strong inhibition factor in these buds[16]. In our experiments, all nodal explants belonged in the second group. The flowering shoots of miniature roses are shorter and more compact than those of hybrid tea roses or the branches of climbing roses. Thus, the basipetal gradient inhibition in the lateral buds of miniature roses caused by apical dominance is not as strong as in the lateral buds of hybrid tea roses

or climbing roses. Results showed that the growth and development of lateral buds were most affected by the amount of nodal segments rather than the node position of lateral buds (Table 1 and 3). It is possible that the poor growth of the lowest two lateral buds resulted from a limitation of parental stem tissue rather than inhibition by apical dominance. Consequently, these two dormant buds would not be recommended for tissue culture.

The range of PPF used to induce axillary shoots in rose tissue culture is from 5 (about 400 lux) to 50 μmol $m^{-2}s^{-1}$[1,6,15]. Davies found that higher light intensity (1000 lux) induced larger leaves, but the proliferation rate of shoots was poorer than at a lower light intensity (400 lux)[4]. Bressan et al. reported that shoot proliferation under 66 μmol $m^{-2}s^{-1}$ was equivalent to that under 17 μmol $m^{-2}s^{-1}$[3], however, under the former environment, shoots were stunted and contained many senescent leaves. Moreover, shoot multiplication was the greatest in shoots incubated under 148 μmol $m^{-2}s^{-1}$, although resulting shoots exhibited an even higher degree of leaf senescence. Khoshi-Khui and Sink showed that shoots grew rapidly for the 1st week under 3000 lux (about 40 μmol $m^{-2}s^{-1}$), although shoots rapidly became chlorotic[9]. Our results revealed that PPFs from 5 to 40 μmol $m^{-2}s^{-1}$ did not affect rate or sprouting explants or the number of multiple shoots, but did affect growth and development of lateral shoots. Under the highest PPF tested, the lateral shoots grew and aged more quickly than those under low PPF (Table 4) Consequently, a PPF of 20 μmol $m^{-2}s^{-1}$ was optimum for inducing lateral shoots from nodal segments in a 4-week period. Our findings were similar to those (optimum PPF was 17 μmol $m^{-2}s^{-1}$ in a 4-week culture) of Bressan et al.[3].

Cytokinin was essential for multiple shoot formation from cultured shoot tips of the climbing rose, 'Improved Blaze'. Furthermore, indoleacetic acid (IAA) neither enhanced nor repressed shoot multiplication regardless of BA concentration[8]. Our results showed that shoot proliferation in vitro from nodal segments was largely resulted from BA concentration rather than NAA. Largely the result of BA concentration with NAA having little effect. However, the optimal BA concentration for shoot proliferation was cultivar specific and depended on the types of explant tissue. Bressan et al. discovered that inclusion of BA at 0.03 to 0.3 mg l^{-1} in the medium stimulated development of 'Gold Glow' buds, yet buds of 'Improved Blaze' and 'Mr. Lincoln' developed rapidly in the absence of BA[3]. Short et al. found that a similar response in bud development of *Rosa arvensis* or miniature rose 'Scarlet Gem' incubated on media containing NAA at 0.1 mg l^{-1} plus BA at 0.2 or 1.0 mg l^{-1}, although BA at 0.2 mg l^{-1} produced more vigorous shoots than at 1.0 mg l^{-1}[14]. In our studies, buds of 'Red Sunblaze' responsed to all BA concentrations (Table 5), and results from the other five cultivars agreeded with those obtained with 'Scarlet Gem'[14]. Yet, 'Royal Sunblaze' (Table 5) and 'Lavender Jewel' responded differently. Shoots of 'Royal Sunblaze' aged more quickly than those of 'Red

Sunblaze' (Table 4), especially at a BA concentration of 0.1 mg l^{-1} or less. These reuslts support the effect of cytokinins delaying senescence and increasing nutrient sink activity[12]. Increasing BA concentration up to 1 mg l^{-1} not only delayed shoot aging of 'Royal Sunblaze,' but also enhanced development of multiple shoots from nodal explants (Table 5).

Labor is by far the largest cost involved in micropropagation at the present time, accounting for 60% on up to 85% in extreme cases[5]. Improving the efficiency of aseptic operation is important to minimize production costs. General procedures of establishing nodal explants aseptically have included recutting the end of the shoot that may have absorbed bleach before the remainder of the shoot is explanted[15]. Our results revealed that the recut was not only unnecessary, but also increased the cost of labor. In summary, the most efficient production of the tested miniature rose cultivars included a cultural density of 9-16 complete nodal segments oriented vertically or inclined on a medium containing BA at 0.1 or 1.0 mg l^{-1} under a PPF of 20 μmol $m^{-2}s^{-1}$1.

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References

1. Barve, D. M., R. S. Iyer, S. Kendurkar and A. Mascarenhas. 1984. An effective method for rapid propagation of some budded rose varieties. Indian J. Hortic. 41: 1～7.
2. Bhojwani, S. S., K. Mullins and D. Cohen. 1987. Micropropagation of *Feijoa sellowina* Berg.. Acta Hortic. 212: 69～76.
3. 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. Hort. Sci. 107: 979～990.
4. Davies, D. R. 1980. Rapid propagation of roses in vitro. Scientia Hortic. 13: 385～389.
5. Donnan, A. Jr. 1986. Determining and minimizing production costs. In Current Plant Science and Biotechnology in Agriculture, edited by Zimmerman, R. H., R. J. Griesbach, F. A. Hammerschlag and R. H. Lawsoin. pp. 167～174. Martinus Nijhoff Publishers.
6. Douglas, G. C., C. B. Rutledge, A. D. Casey and H. S. Richardsoin. 1989. Micropropagation of floribunda, ground cover and miniature roses. Plant Cell Tissue and Organ Culture 119: 55～64.
7. 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 soft cuttings. Scientia Hortic. 35: 293～299.
8. Hasegawa, P.M. 1980. Factors affecting shoot and root initiation from cultured rose

shoot tips. J. Amer. Soc. Hort. Sci. 105: 216～220.
9. Khosh-Khui, M. and K. C. Sink. 1982. Micropropagation of new and old world rose species. J. Hort. Sci. 57: 315～319.
10. MacDonald, B. 1986. Practical woody plant propagation or nursery growers. pp. 595～632. Timber Press, Oregon.
11. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Phsiol. Plant 15: 473～497.
12. Salisbury, F. B. and C. W. Ross. 1985. Hormones and growth regulators: cytokinins ethylene, abscisic acid, and other compounds. pp. 330～349. In Plant Physiology. Third edition. Wadsworth, Inc. pp. 330～349.
13. Sharma, K. K. and T. A. Thorpe. 1990. In vitro propagation of mulberry (*Morus alba* L.) through nodal segments. Scientia Hortic. 42: 307～320.
14. Short K. C., L. Price and A. V. Roberts. 1981. Micropropagation of roses. The Rose Annual. Edited by J. Harkness. pp. 138～144. The Royal National Rose Society. London.
15. Skirvin, R. M., M. C. Chu and H. J. Young. 1990. Rose. In Handbook of Plant Cell Culture. Vol. 5, Ornamental Species. Edited by Ammirato, P.V., D. A. Evans, W. R. Sharp and Y. P. S. Bajaj. pp. 716～743. McGraw-Hill, Inc.
16. Zamski, E., S. Oshri and N. Zieslin. 1985. Compartive morphology and anatomy of axillary buds. Bot. Gaz. 146: 208～212.
17. Zieslin, N. and A. H. Halevy. 1976. Flower bud atrophy in 'Baccara' roses. III. Effect of leaves and stems. Scientia Hortic. 4: 73～78.