### MASS PROPAGATION OF THE DWARF ROSE CULTIVAR 'VICTORY PARADE'

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Abstract. The dwarf rose 'Victory Parade' meristem culture was initiated and propagated on the Murashige, Skoog (M. S.) basal medium supplemented with benzyladenine (BA;  $1.0 \text{ mg} \cdot 1^{-1}$ ), indolylbutyric acid (IBA;  $0.001 \text{ mg} \cdot 1^{-1}$ ), and ascorbic acid ( $5 \text{ mg} \cdot 1^{-1}$ ). The elongation medium was 1/2 M.S. supplemented with kinetin ( $0.05 \text{ mg} \cdot 1^{-1}$ ), indolylacetic acid (IAA;  $1.0 \text{ mg} \cdot 1^{-1}$ ), and ascorbic acid ( $5 \text{ mg} \cdot 1^{-1}$ ). Woody plant medium (WPM) supplemented with IAA ( $0.2 \text{ mg} \cdot 1^{-1}$ ) was the most effective for rooting. Roots could be initiated after the transfer of plantlets from the elongation or proliferation medium to an unsterile substrate soaking them for 16 h in 1/3 WPM supplemented with IBA ( $25 \text{ mg} \cdot 1^{-1}$ ) or moistening the substrate with 1/3 WPM supplemented with IBA ( $10 \text{ mg} \cdot 1^{-1}$ ).

Key words: rose, meristem culture, micropropagation, rooting.

## INTRODUCTION

Micropropagation is becoming increasingly important for commercial propagation of roses because of its rapidity and potential for planned production. The establishment of tissue culture propagation systems for different species and cultivars of roses has been described by Hasegawa (1979), Bressan et al. (1982), Douglas et al. (1989), and Campos & Pais (1990).

In our study the sterile tissue culture procedure for the propagation of the dwarf rose cultivar 'Victory Parade' was combined with unsterile rooting.

# MATERIAL AND METHODS

The sterile culture for the propagation of the dwarf rose 'Victory Parade' was established from greenhouse-grown two-year-old plants.

Rose shoots were cut into approximately 2—3-cm-long sections. They were surface-sterilized by rinsing for 30 s in 96% ethanol. It was followed by vigorous stirring for 20 min in a filtered solution of calcium hypochlorite (6% w/v) supplemented with a few drops of Tween 80. Then the shoots were rinsed three times in sterile distilled water. After the surface sterilization approximately 1-cm-long sections with one bud were excised from these cuttings. These were placed horizontally into the promoting medium (Table 1). In the rooting experiments we used besides the Murashige and Skoog (M.S.) (1962) basal medium the Woody Plant Medium (WPM) (Lloyd & McCrown, 1981). The formulation of this and other media is presented in Table 1. Plantlets were cultured in glass jars capped with aluminium foil.

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Table 1

#### Formulation of media

	Medium					
Components	Promot-	Promot- ing Prolif- eration	Elon- gation	Rooting		
	ing			M.S.	WPM	
M.S.	1/1	1/1	1/9	1/2	ндоцьных адодьных	
micronutrients	1/1 1/1	1/1 1/1	1/2 1/1	1/3	1/1	
WPM macronutrients without K <sub>2</sub> SO <sub>4</sub> suppl KNO <sub>2</sub> 800 mg · 1-1	разинцы м действия всех бе копление д	в содица света и в исключ итп <u>и</u> даци	нанинетина кинетина нения сей Поста Соб	na gan na ga Hangan na si Hili 21-ma X Pranggara	1/1	
FeEDTA. $mg \cdot 1^{-1}$	40	40	40	40	40	
Ascorbic acid, $mg \cdot 1^{-1}$	5	5	5	_	_	
BA, $mg \cdot 1^{-1}$	1.0	1.0	a (1977) 30	CARC TRATILITY	10124-503-509	
Kinetin, $mg \cdot 1^{-1}$	and starting the second	1-10030-3410	0.05	an support of	ALC: NUMBER OF	
IAA, $mg \cdot 1^{-1}$	are nily bar	againd dive	1.0	0.2	0.2	
IBA, $mg \cdot 1^{-1}$	0.001	0.1		0.1	20010 2010	
Sucrose, $g \cdot 1^{-1}$	30	30	30	30	30	
Agar, g·1 <sup>-1</sup>	8	8	8	8	8	

Cultures were maintained in phytotron at  $23\pm2$  °C with a 16 h photoperiod under cool white lamps. The light intensity was about 1000 lx. Acclimatization was provided on perlite under a polyethylene tent in phytotron.

## RESULTS

After three weeks on the initial medium, 0.5–0.8 cm plantlets were obtained. These were transferred to the proliferation medium. After four weeks on the proliferation medium, 1.0–1.5 cm plantlets were got. The multiplication rate was about 6–8. These plantlets rooted on the rooting medium but they were too short for acclimatization. Therefore it was necessary to use an elongation stage between proliferation and rooting. After four weeks the length of elongated shoots was 2.5–4 cm and the plantlets were transferred to the rooting medium (1/3 M.S. or WPM) for 10 days. When plantlets were grown on the rooting medium for more than 15 days the rooting percentage increased but roots blackened and turned brittle. Such results were described earlier by Campos & Pais (1990).

Table 2

Rooting medium	No. of	Rooted Not rooted	Rooting %	Acclimatization	
	plantlets			Survived plants	Survival %
WPM	1950	$\frac{1630}{320}$	83.6	1602 309	98.2 96.5
1/3 M.S.	325	100 225	30.8	83 208	83.0

Rooting in vitro (10 days) and acclimatization

The rooting of plantlets was better on the WPM than on the M.S. medium (respectively 83.6% and 30.6% visible 1—5 mm roots). However, the difference in plants surviving after acclimatization was not so big (Table 2).

Part of the plantlets ( $\approx 30\%$ ) formed roots already on the elongation medium (459 rooted plants from 1517). If these plantlets were transferred to perlite without treatment with IBA, the survival percentage was insufficient ( $\approx 50\%$ ). When treated with IBA solution (1/3 M.S. + 25 mg \cdot 1^{-1} IBA + 10 mg \cdot 1^{-1} citric acid; 16 h) all the plants survived (Table 3).

Table 3

Plantlets rooted on the elongation medium (40 days) and then acclimatized

No. of plantlets	Treating with IBA	Survived plants	Survival %
229	shoots	136	59.4
230	+	230	100.0

The plantlets not rooted on the elongation medium (1059) were treated in different ways. One part was soaked in 1/3 M.S. + 25 mg  $\cdot 1^{-1}$  IBA + 10 mg  $\cdot 1^{-1}$  citric acid for 20 min or 16 h. The difference in the rooting of plantlets soaked for 20 min or 16 h in M.S. was not significant. However, the rooting and survival of plantlets increased after soaking in WPM (Table 4).

Another part of the plantlets was planted directly into perlite. After planting the perlite was moistened once with one of the following solutions: 1/3 M.S. + 10 mg·1<sup>-1</sup> IBA; 1/3 M.S. + 10 mg·1<sup>-1</sup> IBA + 10 mg·1<sup>-1</sup> citric acid; 1/3 WPM + 10 mg·1<sup>-1</sup> IBA; or 1/3 WPM + 10 mg·1<sup>-1</sup> IBA + 10 mg·1<sup>-1</sup> citric acid. Rooting was about 50% better when the moistening solution included citric acid. Like in the soaking experiments, the moistening of perlite with WPM gave somewhat better results than the moistening with M.S. (Table 4).

Table 4

Plantlets grown on the elongation medium (40 days) and then rooted in perlite

Treatment of shoots	No. of shoots	No. of rooted shoots	Survival %
Soaking in:	Id		
$1/3 \text{ M.S.} + 25 \text{ mg} \cdot 1^{-1} \text{ IBA} + 10 \text{ mg} \cdot 1^{-1} \text{ CA}; 10 \text{ min}$	160	115	71.9
1/3 M.S.+25 mg · 1 <sup>-1</sup> IBA+10 mg · 1 <sup>-1</sup> CA; 16 h	150	110	73.9
WPM+25 mg $\cdot 1^{-1}$ IBA+10 mg $\cdot 1^{-1}$ CA; 16 h	138	109	79.0
Moistening perlite with:			
$1/3 M.S. + 10 mg \cdot 1^{-1} IBA$	175	75	49.9
$1/3$ M.S. + 10 mg $\cdot$ 1 <sup>-1</sup> IBA + 10 mg $\cdot$ 1 <sup>-1</sup> CA	140	106	75.7
$1/3 \text{ WPM} + 10 \text{ mg} \cdot 1^{-1} \text{ IBA}$	155	69	44.6
$1/3$ WPM+10 mg $\cdot 1^{-1}$ IBA+10 mg $\cdot 1^{-1}$ CA	140	119	85.0

CA, citric acid.

On the proliferation medium 1–1.5 cm (occasionally up to 2 cm) long shoots were formed. We selected shoots longer than 1.5 cm, separated the lower leaves, leaving only the upper 3–4 leaves on the shoot (smaller shoots may be transplanted to the elongation medium). These longer shoots were planted onto perlite after soaking solutions with IBA or perlite moistened with solutions containing IBA. After these procedures about 84% of the shoots rooted and survived. On the proliferation medium clusters of shoots (4–7 shoots in each) developed. When these clusters were soaked in WPM solution supplemented with 25 mg  $\cdot 1^{-1}$  IBA and then transferred to perlite, 69% of the shoots rooted. After 30 days the clusters were divided into individual plants (Table 5).

Table 5

Treatment of shoots	No. of shoots	No. of rooted shoots	Survival %
Control	100	9	9.0
Soaking 16 h in:			
$1/3 \text{ M.S.} + 25 \text{ mg} \cdot 1^{-1} \text{ IBA} + 10 \text{ mg} \cdot 1^{-1} \text{ CA}$	180	130	72.2
$1/3 WPM + 25 mg \cdot 1^{-1} IBA + 10 mg \cdot 1^{-1} CA$	153	128	84.4
$1/3 \text{ WPM} + 25 \text{ mg} \cdot 1^{-1} \text{ IBA} + 10 \text{ mg} \cdot 1^{-1} \text{ CA}$	55	38	69.1
	(10 clusters)		
Moistening perlite with:			
$1/3$ M.S. + 10 mg $\cdot$ 1 <sup>-1</sup> IBA + 10 mg $\cdot$ 1 <sup>-1</sup> CA	240	129	53.7
$1/3$ WPM + 10 mg $\cdot$ 1 <sup>-1</sup> IBA + 10 mg $\cdot$ 1 <sup>-1</sup> CA	128	93	72.7
CA, citric acid.			

Plantlets grown on the proliferation medium (40 days) and rooted in perlite

CA, citric acid. Frequently it was necessary to keep the plantlets for some time in culture vessels before planting them to perlite or to other substrates. As a rule, the plantlets were kept in cold  $(2-5^{\circ}C)$  under a low light intensity. In the case of 'Victory Parade' it is possible to keep the plantlets for a least three months on the elongation medium under a low light intensity (80—120 lx) in phytothrone (20°C). Although the lower leaves turned yellow and roots blackened, the plantlets did not die (in a few days) as described by Campos & Pais (1990). When yellow leaves and black roots were removed and shoots were treated with solutions containing IBA, most of the plants survived after acclimatization.

#### DISCUSSION

Douglas et al. (1989) stated that the in vitro rooting of (cv. Queen Elizabeth) shoots smaller than 2 cm was low and attempts to achieve rooting by transferring shoots directly to compost were generally unsatisfactory. Rooting in compost increased 84-100% in case of two-week pretreatment in sterile conditions on the medium containing  $0.1 \text{ mg} \cdot 1^{-1}$  IAA. In our experiments we achieved up to 84% rooting of small shoots with treating them with solutions containing IBA.

A combination of the tissue culture method with unsterile rooting of shoots on perlite or some other substrate cut the price of production, as no expensive equipment or highly qualified workers were required. It also reduced the amount of chemicals etc. necessary. Campos, P. S. and Pais, M. S. S. 1990. Mass propagation of the dwarf rose cultivar 'Rosamini'. — Sci. Hort., 43, 321—330.

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#### MINIROOSI SORDI 'VICTORY PARADE' MASSPALJUNDUS

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Miniroosi 'Victory Parade' meristeemkultuuri stardi- ja paljundussöötmeks oli Murashige ja Skoogi (M.S.) põhisööde, millele oli lisatud bensüüladeniini (1,0 mg/l), askorbiinhapet (5 mg/l) ja indolüülvõihapet (IBA; 0,001 mg/l). Pikendussööde oli 1/2 M.S. + kinetiin (0,05 mg/l) + indolüüläädikhape (IAA; 1,0 mg/l) + askorbiinhape (5 mg/l). Juurutamiseks oli sobivaim puittaimede sööde (WPM) + IAA (0,2 mg/l). Toodangu odavdamiseks on masspaljunduses võimalik ära jätta steriilne juurutusfaas ja viia paljundus- või pikendussöötmel kasvanud võrsed otse mittesteriilsele substraadile, töödeldes neid enne 16 tundi 1/3 WPM + IBA (25 mg/l) või kastes pärast istutamist 1/3 WPM + IBA (10 mg/l) lahusega.

#### МАССОВОЕ РАЗМНОЖЕНИЕ МИНИАТЮРНОЙ РОЗЫ 'ВИКТОРИ ПАРАДЕ'

#### Раел ВАРДЬЯ, Тынис ВАРДЬЯ

Меристемную культуру миниатурной розы 'Виктори Параде' получили на среде Мурасиге—Скуга в присутствии бензиладенина (1,0 мг/л), индолилмасляной кислоты (ИМК 0,001 мг/л) и аскорбиновой кислоты (5 мг/л). Удлинительная среда: 1/2 среды Мурасиге—Скуга + кинетин (0,05 мг/л) + индолилуксусная кислота (ИУК) 1,0 мг/л + аскорбиновая кислота (5 мг/л). Среда для укоренения: среда древесных растений (WPM) + ИУК (0,2 мг/л).

Для массовых размножений не обязательно использовать стерильную фазу укоренения. Растения можно сажать на нестерильный субстрат непосредственно со среды умножения или удлинения, обрабатывая их до высадки в течение 16 ч раствором 1/3 WPM + ИМК (25 мг/л) или поливая субстрат после посадки раствором 1/3 WPM + + ИМК (10 мг/л).