FACTORS CONTROLLING ADVENTITIOUS ROOT FORMATION ON STEM EXPLANTS OF ROSE (*Rosa hybrida* CV. 'MOTREA') *IN VITRO*

R.L.M. Pierik; P.A. van de Pol.

Department of Horticulture, Agricultural University, Haagsteeg 3, 6708 PM Wageningen, The Netherlands.

RESUMEN. Factores que controlan la formación de raíces adventicias sobre explantes de tallos en rosa (**Rosa hybrida** cv. 'Motrea') **in vitro.**

Los factores que afectan la formación de raíces adventicias fueron estudiadas en explantes de tallo en rosa florecientes (**Rosa hybrida** cv. 'Motrea').

En enraizamiento de la parte basal terminal de segmentos de tallos fue un genotipo dependiente y generalmente ocurrió óptimamente en explantes de tallos los cuales fueron colocados hacia abajo sobre el medio de cultivo.

La continua obscuridad fue importante para el enraizamiento, aunque la luz durante los primeros días del período de cultivo promovieron el enraizamiento en comparación con una continua obscuridad. En el rango de 21-23-25°C solamente un pequeño efecto de temperatura fue observado. Buena formación de raíces ocurrió solamente cuando ambos azúcar y macrosales estuvieron presentes en el medio. La formación de raíces adventicias fue mucho mejor sobre un medio con glucosa que con sacarosa, el agar blando jugó un papel muy importante durante el enraizamiento. Las auxinas fueron un absoluto requerimiento para el enraizamiento, pero este fue solamente necesario durante las primeras 24 horas del cultivo **in vitro**.

PALABRAS CLAVE: Formación de raíces adventicias, micropropagación, Motrea, **vitro**, tallos, **Rosa hybrida**, rosa, explantes.

SUMMARY. Factors effecting adventitious root formation were studied in stem explants of flowering rose (**Rosa hybrida** cv. 'Motrea').

Rooting at the basal ends of the stem segments was genotype dependent and usually occurred optimally in stem explants which were placed upside down on the culture medium.

Continuous darkness was important for rooting, although light during the first days of the culture period promoted rooting in comparison to continuous darkness. In the range 21-23-25°C only a small effect of temperature was observed. Good root formation occurred only when both sugar and macrosalts were present in the medium. Adventitious root formation was much better on a culture medium with glucose than with sucrose. The agar brand played a very important role during rooting. Auxin was an absolute requirement for rooting and was only necessary during the first 24 hours of in vitro culture.

KEY WORDS: Adventitious root formation, micropropagation, Motrea, vitro, stem, **Rosa hybrida**, rose, explants.

Abbreviations: MS - Macrosalts according to Murashige and Skoog (1962). IBA - 4(3-Indolyl)butyric acid, potassium salt, MNR - Mean number of roots per explant, % R - Percentage of rooting.

INTRODUCTION

In The Netherlands the rose is the most important cut flower with an auction turnover of 640 million U.S. dollars in 1994. Rose, a perennial woody shrub, is usually propagated vegetatively. The traditional cloning systems are: making of shoot cuttings or grafting of a cultivar on a suitable rootstock. In recent years new propagation systems have been developed at the Agricultural University in Wageningen, The Netherlands: stenting (grafting a piece of stem with a bud and leaf of a cultivar on a piece of stem of a rootstock, which has to be rooted), and micropropagation (Marcelis-Van Acker, 1994; Van de Pol and Breukelaar, 1982; Van de Pol and Pierik, 1995).

In most propagation systems the formation of adventitious roots at the basal ends of the stems is an essential process. Since also in micropropagation

regeneration of roots plays an essential role, we decided to study adventitious root formation in a newly developed model system: excised stem explants on a well defined culture medium in vitro (Van der Krieken *et ald.* (1993). This system will help us to gain a better insight into the plant, nutritional and physical factors determining the formation of adventitious roots.

MATERIAL AND METHODS

In almost all experiments *Rosa hybrida* 'Motrea' was used to study adventitious root formation. Rose plants were grown in a heated greenhouse so that plant material was available year- round. Material was selected (homogeneous, same diameter and developmental stage) among young axillary shoots from buds positioned directly below the flower buds.

After removal of the leaves and shoot tip, the upper two elongating internodes were sterilized as follows: dipped for a few seconds in alcohol 70% (v/v), 20 minutes in 1% NaOCl (with a few drops of Tween 20), and rinsed three times (for 3, 5, and 15 minutes respectively) in sterile tap water.

Sterilized cylindrical stem segments, 3 mm in length and without bud, were cut aseptically in a laminar airflow cabinet, and subsequently transplanted on the culture media with their basal ends up. Explants between nodes and from different stems were always at random divided over de various Petri dishes and treatments. Each treatment consisted of 4 Petri dishes (each dish was an experimental unit) with 6 stem explants each. Explants were grown at regular distances from each other. Petri dishes (diameter 6 cm) contained 15 ml of medium, and were sealed with Parafilm.

The following basic culture medium was used: MS macrosalts full strength, MS microsalts full strength (exept Fe), NaFeEDTA 37.5 mg/l, glucose 45 g/l, IBA 2.0 mg/l, Vitamin B1 0.4 mg/l, méso- inositol 100 mg/l, agar 7 g/l (MC 29 from Lab M, Amersham, England), pyrex-distilled water, at a pH 5.8 before autoclaving. Media were sterilized in an autoclave at 121°C.

Unless otherwise stated, stem segments were incubated in a growth chamber at 23°C in complete darkness. Occasionally light periods were given under a schedule of 16 hours photoperiod provided by fluorescent tubes (Pope, FDT/58W, 84HF, 8-10 WM^2).

All rooting experiments usually had one variable factor (cultivar, type of plant material, nutritional, hormonal, physical, etc.). Standard (control) treatments, as described in material and methods, are in the tables indicated with an asterisk (*).

After 4 weeks of in vitro culture rooting was evaluated by determining the % R and the MNR over all explants, except infected ones. Only those roots could be counted that were visible as root initials or as elongated roots.

RESULTS

Before using the experimental system described in material and methods, quite a number of rooting experi-

TABLE 1. **The influence of the strength of the MS-macro-salts (A), the sugar concentration (B), and the agar brand (C) on adventitious root formation of stem segments of** Rosa hybrida **'Motrea' in vitro. Agar 1, Becton Dickinson purified; agar 2, Becton Dickinson Grade A; agar 3, Difco Bacto agar; agar 4, MC 29 from Lab M, Amersham, England. All agars concentrations 0.7%.**

Factors examined	Conc.	% R	MNR
A MS-macrosalts (strength)	0	25	0.9
	0.25	48	1.9
	0.50	52	1.1
	0.75	92	4.5
	1.00*	96	6.5
	1.25	89	3.3
	1.50	33	1.4
B Glucose (%)	0	0	0
	2.5	100	3.5
	3.5	96	9.9
	4.5*	100	11.2
	5.5	88	8.8
	6.5	96	10.0
	7.5	96	9.7
Sucrose (%)	0	0	0
	2.5	0	0
	3.5	0	0
	4.5	79	2.1
	5.5	96	3.2
	6.5	96	4.0
	7.5	96	4.1
	8.5	92	4.6
	9.5	88	4.2
	10.5	75	2.9
C Agar brand nr.	1	46	1.8
	2	92	4.5
	3	94	6.0
	4*	96	7.5

ments were done to examine limiting factors for the regeneration of adventitious roots of the rose 'Motrea' The composition of the medium and all other conditions came into existence after numerous preliminary trials in recent years.

After isolation, first of all swelling of the tissue started and callus was rapidly formed. This was followed by root formation, mainly at the basal side of the explants above the medium. This indicated that polarity of root regeneration remained intact, even when the explants were placed upside down on the medium. Occasionally also some roots were formed at the upper part of the explant (in the medium), be it not abundantly. The first visible adventitious roots were detected after 7-8 days, indicating that the rooting process was very rapid. Four weeks after the start of the experiments, the increase in the percentage of rooting (%R) and the mean number of roots (MNR) had almost stopped.

Comparison of 7 MS-macrosalts concentrations (Table 1 A) showed that rooting increased by raising the concentration of the macrosalts from 0-1.0 strength, with a clear optimal response at 1.0 strength, whereas rooting decreased from 1.0-1.5 strength. At the lowest and highest macro-salt concentrations rooting was very bad.

The role of the glucose and sucrose concentration was examined in a broad concentration range, for glucose 0-7.5% and for sucrose from 0-10.5% (Table 1 B). The concentration range for sucrose was choosen longer, because we expected a rooting optimum at a relatively high concentration of sucrose in comparison with glucose. Without sugar no rooting occurred and all explants died, indicating that sugar was an essential prerequisite for rooting in rose. In the range 3.5-7.5% glucose, rooting is stable and optimal, whereas rooting on sucrose is optimal and stable in the range 5.5-9.5%. Finally it can be concluded that glucose was a much better sugar to induce rooting than sucrose (Photo 1); this photo also shows that root initiation and root length in segments grown on glucose is much better than on sucrose.

The effect of the agar brand (Table 1 C) is remarkable. Agar 1 was very bad for rooting, whereas agar 4 was clearly the best. The rooting on agars 2 and 3 were in between agar 1 and 4. The conclusion from this experiment was that the choice of the agar brand is extremely important. Due to the results shown in Table 1 C, we decided to use only agar 4 in all our in vitro experiments with rose.

TABLE 2. The influence of the IBA concentration on adventitious root formation of stem segments of Rosa hybrida **'Motrea' in vitro.**

Factor examined	Conc.	% R	MNR
IBA conc. (mg/l)	0	0	0.0
	0.25	100	5.3
	0.50	100	6.0
	0.75	100	5.3
	1.00	100	6.0
	2.00*	92	4.5
	3.00	70	2.1
	4.00	33	0.5
	5.00	11	0.1

The influence of the IBA concentration (Table 2) on rooting showed that in the concentration range 0.25-

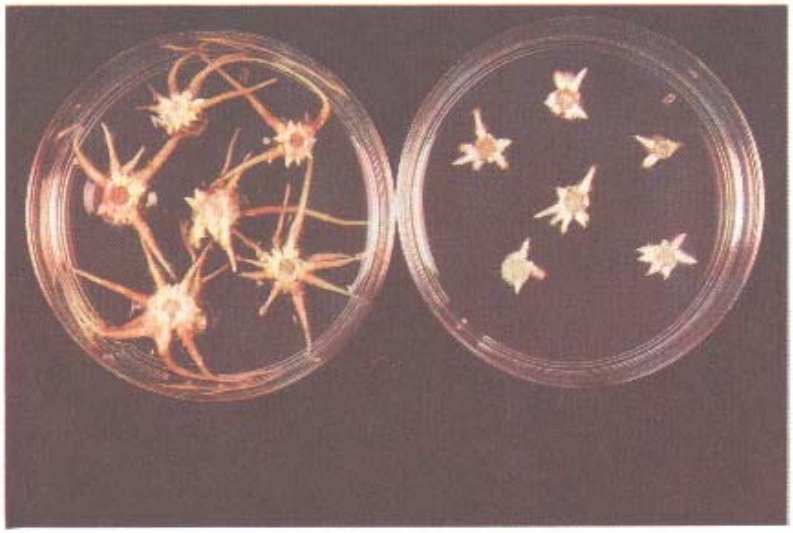

Photo 1. The influence of the glucose concentration (4, 5%, left), and the sucrose concentration (5, 5%, right) on adventitious root formation of rose stem explants in vitro. Rooting on glucose is much better than on suc rose. For further explanation see Table 1 B and text.

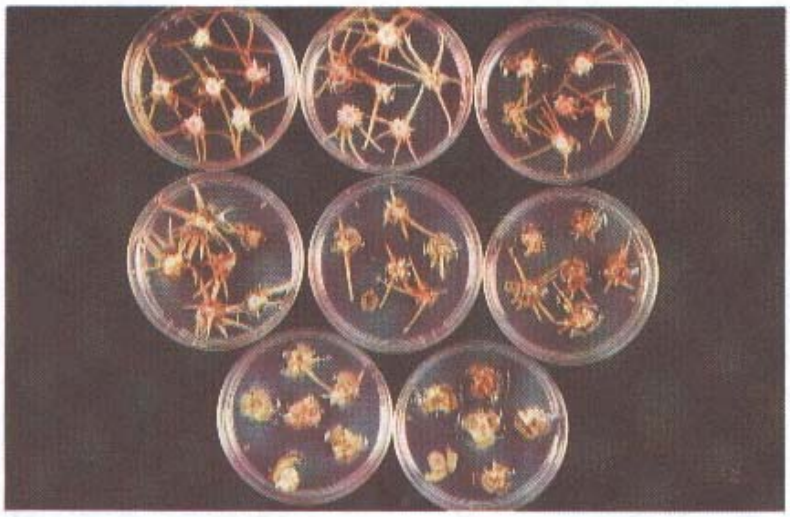

Photo 2. The effect of the IBA concentration on adventitious root formation of stem explants of rose in vitro. **From left to right and from top to bottom: 0.25, 0.50, 0.75, 1.00, 2.00, 3.00, 4.00 and 5.00 mg/l IBA. Without auxin no rooting occurred (see also Photo 3).**

1.00 mg/l rooting was optimal and rather stable, whereas rooting decreased in the range 1.0-5.0 mg/l IBA. No rooting occurred on a medium without auxin, indicating that there is an absolute auxin requirement (Photo 2)

Since auxin was essential for rooting the length of the period in which explants received auxin was varied (Table 3). Explants were initially grown on medium containing 2 mg/l IBA, and tranferred to auxin-free medium after various periods. In the range from 0- 24 hours initial auxin supply, there was increase in rooting and periods of 24 hours or longer give the highest response. This means that an auxin treatment during 24 hours was already enough to induce optimal rooting (Photo 3), whereas permanent auxin slightly inhibited rooting.

TABLE 3. The influence of the duration of the IBA gift on adventitious root formation of stem segments of Rosa hybrida **'Mòtrea'. IBA treatment (4 mg/l) was given immediately after isolation. After the IBA treatment segments were transferred to a new medium without IBA.**

Factor examined	Duration (hours)	% R	MNR
Number of hours auxin treatment	0	0	0.0
	6	95	2.9
	12	75	6.5
	24	92	6.5
	36	96	7.5
	48	83	6.0
	144	100	7.9
	672*	100	6.7

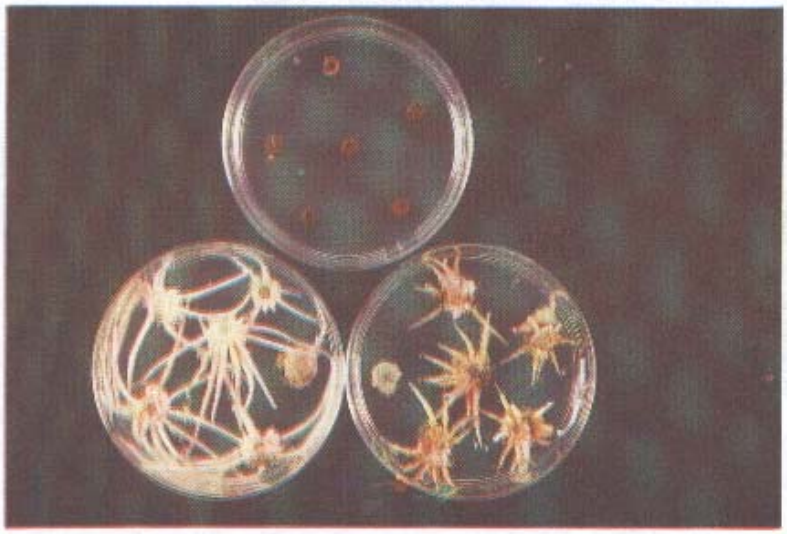

Photo 3. The influence of the auxin IBA (2 mg/l) on rooting of rose segments. Upper Petri dish: no auxin treatment (no rooting). Bottom dish, left: 2 days auxin, followed by 26 days auxin-free medium. Bottom dish, right: continuous (28 days) auxin in the medium.

The influence of light/darkness on rooting was examined by giving an initial dark period (0, 2, 4, 6, 8 and 28 days), followed by a light period (28, 26, 24, 22, 20 and 0 days) respectively. Table 4 A shows that continuous darkness resulted in a much better rooting than continuous light. However, when the initial dark period was increased from 0-8 days rooting was strongly decreased. However, continuous darkness remained optimal. The result of this experiment was the reason to do all experiments in darkness.

TABLE 4. The influence of an initial dark period followed by light (A), and an initial light period followed by a dark period (B), on adventitious root formation of stem segmens of Rosa hybrida **'Motrea' in vitro.**

Factors examined	Duration (days)	% R	MNR
A Initial dark period	0	100	4.6
	2	88	3.8
	4	88	2.4
	6	96	2.2
	8	63	1.2
	28*	96	9.9
B Initial light period	0*	100	4.5
	7	97	5.6
	14	72	3.0
	21	80	2.6
	28	91	2.5

The influence of light and darkness was also examined by giving an initial period of light (0, 7, 14, 21 and 28 days), followed by a dark period (28, 21, 14, 7 and 0 days) respecti-vely. In this experiment (Table 4 B) an initial light period of 7 days, followed by darkness appeared to be optimal for rooting of rose in comparison with all other treatments. This means that there was in the first week a promoting effect of light and and a slightly inhibiting effect of darkness (Photo 4).

The role of temperature (Table 5) on rooting was examined in the range 21, 23 and 25°C. Rooting increased from 21-23°C, but from 23-25°C rooting decreased slightly. Due to this result all our experiments were carried out at 23°C.

TABLE 5. The influence of the temperature on adventitious root formation of stem explants of Rosa hybrida **'Motrea'.**

Factor examined	°C	% R	MNR
Temperature	21	96	4.1
	23*	92	7.0
	25	92	6.1

Table 6 A shows that there was a clear cultivar effect on adventitious rooting. Our standard cultivar 'Motrea' rooted much better than 'Madelon', and 'Sonia'. But it is possible that the optimal rooting conditions for 'Motrea' (as used in this research) were not optimal for the cultivars 'Madelon' and 'Sonia'.

Photo 4. The influence of light/darkness on adventitious root formation of rose segments in vitro. **From left to right and from top to bottom: 28 D (continous darkness), 7 L + 21 D, 14 L + 14 D, 21 L + 7 D, and 28 L (continuous light). D means days darkness, followed by L (days light).**

TABLE 6. The influence of 3 rose cultivars (A), the length of the explants, and the way of inoculation (B) on adventitious root formation of stem segments of rose in vitro. BED means basal ends down, and BEU means basal ends up.

Factor examined		% R	MNR
A Cultivar	'Madelon'	83	3.2
	'Sonia'	100	4.0
	'Motrea'*	100	7.2
B Length of the explant (mm) and polarity	3 BED	44	1.3
	5 "	50	1.7
	7 "	85	4.1
	3 BEU*	96	7.1
	5 "	98	6.1
	7 "	100	7.4

Finally Table 6 B clearly demonstrates that placing the segments up-side-down was much better for rooting than positioning the explants with basal ends down. Rooting of explants with basal ends down increased by increasing explant length from 3-7 mm, but this did not occur in explants with basal ends up.

DISCUSSION

Our experiments with rose clearly support the view that a complex of plant and environmental factors determines rooting in vitro. Factors like sugar, macrosalts, auxin, darkness, light and some plant factors influence the rooting process. Most observations in our experiments with rose *in vitro* are in accordance with the literature (Pierik, 1969; Pierik & Segers, 1973), describing results with *Asparagus*, *Gerbera*, *Lunaria*, *Phaseolus*, and *Rhododendron*.

The role of light/darkness in the process of adventitious root formation forms a very puzzling part, because in literature not only inhibition of rooting (Caboni *et al.*, 1992; Drew *et al.*, 1993; Druart *et al.*, 1982; Karhu and Zimmerman, 1993; Olieman-van der Meer *et al.*, 1971; Pierik and Steegmans, 1975; Van der Krieken *et al.*, 1992; Rugini *et al*, 1993) by light was reported, but also promotion (Gautheret, 1969). A reasonable explanation for the diverse effect of light/darkness cannot be given. However, Dumas & Monteuuis (1995) suppose that reduction of the light at the base of shoots provides an environment conducive to the accumulation of photosensitive auxin and/or cofactors in the basal tissues. Van der Krieken *et al.* (1992) showed that the breakdown of IBA in the medium cannot be accounted for by the negative effect of light. But from all the facts it appears that light/darkness is intimately involved in the formation of adventitious roots; it is possible that phytochrome participates in root new formation, as shown by Bertazza *et al.* (1995).

Three hypothetical explanations can be given for the promotive effect of inverting explants on rooting (Pierik and Steegmans, 1975); (1) oxygen supply at the basal part is much better when the explants are inverted; (2) in inverted explants basipetally transported substances accumulate as no diffusion from the basal part into the agar medium can occur; (3) the normal auxin gradient from tip to base is preserved. The first explanation seems to be the most likely one since regeneration of organs has a definite oxygen requirement and under anaerobic conditions (basal ends down) it is unlikely that rooting takes place. The question whether substances beneficial for rooting diffuse (in explants with basal ends down) into the agar remains unanswered. Whether preservation of the auxin gradient in inverted explants takes place, needs further attention.

This research demonstrates clearly that in rose rooting of adult material can be realized optimally under a special set of controlled conditions.

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