

Micropropagation of roses (*Rosa* spp.): The effects of different media on in vitro rooting

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In vitro propagation techniques were studied in 3 different genotypes of roses (*Rosa* spp.) and the effects of two different disinfectants (dichloroisocyanuric acid and sodium hypochlorite) were evaluated. The sterilisation based on dichloroisocyanuric acid was more efficient (32.6% of vital explants) when compared to the sterilisation with sodium hypochlorite (7.3% of vital explants). The sterilised and vital explants were transferred onto a shoot proliferation medium based on MS. The subcultivation period lasted 4 months. From the first genotype, 186 shoots were obtained, from the second 219 and from the third only 88 shoots. Sufficiently developed plants were transferred to three different rooting media which differed in the presence of auxin (IBA), sucrose and MS concentration. The results show, that the rooting medium did not have the same effects on all tested genotypes. The first genotype resulted in 82% rooted plants on the medium with 1/2 MS supplemented with 30 g/L of sucrose and no IBA added. The most successful rooting for second genotype (55.6% rooted plants) was obtained on the rooting medium with full strength MS, supplemented with 30 g/L sucrose and 0.5 mg/L IBA. The third genotype rooted most successfully (65.9% plants with developed roots) on the medium with 1/4 MS supplemented with 30 g/L of sucrose and no IBA added. Rooted plants were transplanted into the substrate and acclimatised in the laboratory (common growth chamber) and in the greenhouse (moist chamber). The acclimatisation in the moist chamber had a positive effect on the number of survived plants (90.9% survival) compared with the acclimatisation in the common growth chamber (68.1% survival).

Key words: rose, *Rosa* spp. micropropagation, *in vitro* propagation, *in vitro* rooting

Roses (*Rosa* spp.) are considered to be among the first domesticated ornamental plants. In recent years, there has been a significant increase in the demand for rose plants. Roses are propagated for three distinct production sectors: greenhouse cut flowers production, pot-grown flowering houseplant sales and home gardening market. Many rose cultivars are propagated asexually by budding or grafting of the desired cultivar on selected rootstocks. The miniature (dwarf) roses are propagated on their own root systems (Kane 2000). There have been numerous attempts to develop micropopagation protocols in order to produce roses that are true-to-type and superior to traditionally propagated rose plants in terms of price, quality, and growth performance (Hasegawa 1979, Davies 1980, Valles 1987, Horn et al. 1988, Skirvin et al. 1990, Nikbakht et al. 2005). Bressan et al. (1982) analysed the role of culture conditions, Hyndman et al. (1982a, b), Kosh-Khui and Sink (1982a and b) studied rooting problems.

The main objective of this study was to investigate different sterilisation procedures used for *in vitro* culture initiation of roses. The second objective was to investigate the effect of different nutrient media on *in vitro* rooting of three different rose genotypes in order to determine the most suitable medium for each genotype. The third objective was to compare two acclimatisation environments: in a common growth chamber (in a laboratory) and in a moist chamber (inside a greenhouse).

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MATERIALS AND METHODS

Plant Material and establishment of *in vitro* culture

Shoots from the first rose genotype were collected on October 15, 2009 from the experimental field Pohorski dvor, which belongs to the Faculty of Agriculture and Life Sciences, Hoče, NE Slovenia. Shoots of the second and the third genotype were collected in Tepanje (a village near Maribor, NE Slovenia) on May 26, 2010. In the laboratory for tissue culture, nodal explants taken from newly formed shoots were dissected with a scalpel and used as a source for explants. Prior to surface sterilisation, shoots were cut into two-node explants about 15 mm in length, leaf blades were removed leaving only a small basal section of petiole (Fig. 1).

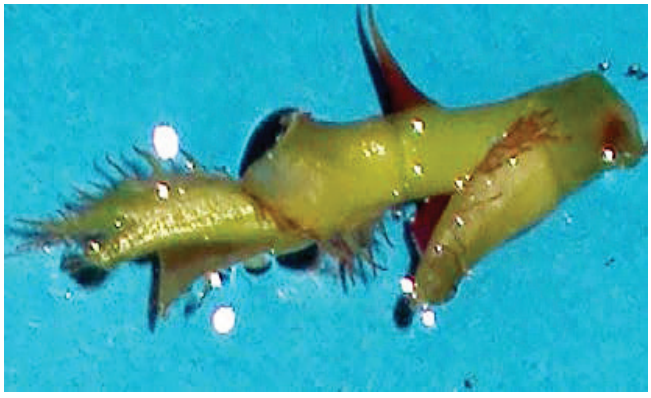

Fig. 1: Two-node explant (Gošnjak 2009)

The sterilisation of explants was based on two approaches (two compounds): dichloroisocyanuric acid (DICA) (Aldrich, Germany) and sodium hypochlorite. For DICA sterilisation, explants were soaked for 20 min in DICA (16.6 g/L) to which 2-3 drops of Tween 20 (a wetting agent) were added and rinsed three times in sterile distilled water. Based on the second approach, the explants were immersed in 70% ethanol for 40 sec, soaked in 1% sodium hypochlorite for 15 min and rinsed three times in sterile distilled water.

Sterilised explants were placed on the medium which contained the following substances: MS (Murashige and Skoog 1962) mineral salts including vitamins (Duchefa M0222, Haarlem, The Netherlands) supplemented with 100.0 mg/L myo-Inositol, 50.0 mg/L citric acid, 50.0 mg/L ascorbic acid, 0.1 mg/L IAA, 0.5 mg/L BAP, 30 g/L sucrose and solidified with 8.0 g/L agar (Plant Agar, Duchefa, Haarlem, The Netherlands). The pH was adjusted to 5.5, before adding gelling agent and autoclaving.

Cultures were maintained in a growth chamber at 23°C with a 15 hours photoperiod (15.000 Lux). Every three days after inoculation, explants were checked for contamination (Table 1). Contaminated and non vital explants were discarded. All vital explants of each genotype were subcultivated every 4 weeks on fresh medium. The number of newly formed shoots and multiplication rates were calculated for each genotype.

ROOTING

In order to induce rooting, *in vitro* developed shoots (1.5-3 cm long) were placed in three special culture media (R1, R2, R3) (Table 2). In the medium R1, 69 shoots of the first genotype, 9 shoots of the second genotype and 44 shoots of the third genotype were placed. In the medium R2, 49 shoots of the first genotype, 10 shoots of the second genotype and 44 shoots of the third genotype were placed. The medium R3 was used only for the first genotype, into which 50 shoots were placed. The cultures were maintained in a growth chamber at 23°C with a 15 hours photoperiod (15.000 Lux). The numbers of rooted plants were determined 24 days after cultivation.

ACCLIMATISATION

All plants with properly developed roots were transferred into the growth substrate (Bio-Potgrond, Klasmann). Before the transfer, roots were carefully washed under tap water in order to remove all remnants of agar. The transferred plants were acclimatised using two approaches. The first acclimatisation approach was based on the use of an ordinary growth chamber in the laboratory whereas the second approach was conducted in a moist chamber inside the greenhouse.

Table 1: Number and percentage of contaminated, non vital and vital explants after sterilisation

Type of sterilisation	Genotype 1		Genotype 2		Genotype 3	
	S1*	S2**	S1*	S2**	S1*	S2**
Number of inoculated explants	34	16	93	88	23	24
Number and percentage of contaminated explants after 26 days	5(44.1%)	2(75.0%)	4(25.8%)	7(53.4%)	3(13.0%)	8(33.3%)
Number and percentage of non vital explants	6(17.7%)	1(6.3%)	6(49.5%)	8(43.2%)	0(0.0%)	6(25.0%)
Number and percentage of sterile and vital shoots	3(38.2%)	3(18.8%)	3(24.7%)	3(3.4%)	0(86.9%)	0(41.7%)

S1*: sterilisation with dichloroisocyanuric acid
S2**: sterilisation with sodium hypochlorite

Table 2: Rooting media R_1, R_2 and R_3

	R1	R2	R3
MS including vitamins	4.4 g/L	1.1 g/L	2.2 g/L
Sucrose	20.0 g/L	30.0 g/L	20.0 g/L
Citric acid	50.0 g/L	/	/
Ascorbic acid	50.0 g/L	/	/
IBA	0.5 mg/L	/	/
Myo-Inositol	100.0 mg/L	100.0 mg/L	100.0 mg/L
Agar	7.0 g/L	8.0 g/L	8.0 g/L
pH	5.7	5.8	5.8

RESULTS AND DISCUSSION

Sterilisation procedure with DICA proved to be better for all three genotypes. In the case of first genotype sterilised with DICA, 38.2% of uninfected plants were obtained. The sterilisation of the second genotype was less successful and only 24.7% explants survived the sterilisation with DICA, whereas the percentage of non vital explants was the highest, reaching 49.5%. We can conclude that the concentration of DICA was too high, or time of treatment was too long for this genotype. The most successful sterilisation was obtained in the third genotype, where 86.9% uninfected and vital explants were obtained. The percentage of contaminated and non vital explants was much higher after the sterilisation with sodium-hypochlorite for all three genotypes (Table 1). Results show that successful induction of *in vitro* culture dependents not only on sterilisation method used but it is also very specific for each genotype. Valles (1987) sterilised explants of 12 different rose genotypes and the number of infected plants depended strongly on the genotype used. In his study, the percentage of infected plants appeared in range from 2.5 to 58.3%. The occurred infections of plant material after chemical sterilisation can be caused by internal infections (micro-organisms present inside the plant itself), which can be reduced with the use of appropriate antibiotics. Other reasons for later occurrence of infections could be inaccurate work (non-sterile forceps or scalpels; working surface not sterilised with 96 % alcohol; not sufficiently sterilised nutrient media; etc.) (Pierik 1998). It is well known, that the growth and proliferation of roses depend strongly on cultivar (Kane 2000). Horn et al. (1988) reported that nodal explants showed better results than shoot tips and that the subcultured shoots with long internodes were more suitable than subcultures from compact shoots with short internodes (in means of growth, number, length and fresh weight of newly obtained shoots).

In our study, higher number of shoots was obtained within the first genotype where the multiplication rate was relatively very high (10.5), and 168 shoots were obtained after 4 month. The multiplication of the second genotype was found to be problematic; a lot of explants died and only 19 shoots were obtained from 26 inoculated explants. Out of 30 explants, 88 shoots were reproduced within the third genotype (Table 3). Carelli and Echeverrigaray (2002) achieved the multiplication rate of 30.3 plantlets per explant of *Rosa hybrida* cv. Baronesse (after 180 days), whereas for six other cultivars the multiplication rate was 1.85-2.88 plantlets per explant (after 60 days).

Table 3: Multiplication rate for three studied genotypes of roses

Genotype	Shoots		Multiplication rate
	Inoculated	Proliferated*	
G1	16	168	10.50
G2	26	19	0.73
G3	*30*	*88*	*2.93*

*after 4 month

Sufficiently developed plants were transferred to three different rooting media, which differed in the presence of auxin (IBA), sucrose and MS concentration. The results show, that the best rooting medium was very specific for each studied genotype. The results obtained by Horn et al. (1988) show that success of *in vitro* rooting of roses is strongly dependent on cultivar. The first genotype developed 82% of rooted plants on the medium with 1/2 MS supplemented with 30 g/L of sucrose and no IBA added after 24 days. The most successful rooting for second genotype (55.6% plants with developed roots) was obtained on the rooting medium with full strength MS, supplemented with 30 g/L sucrose and 0.5 mg/L IBA. The third genotype rooted most successfully (65.9% plants with developed roots) on the medium with 1/4 MS supplemented with 30 g/L of sucrose and no IBA added (Fig. 2).

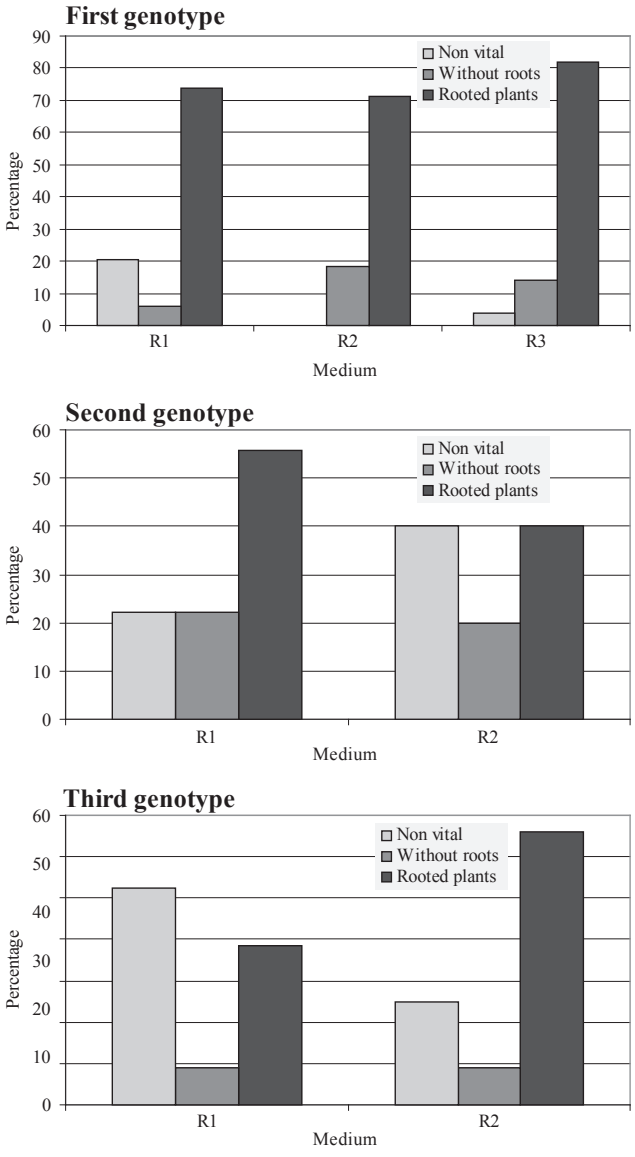

Fig. 2: Percentage of rooted, non rooted and failed shoots for three rose genotypes on different rooting media

The rooted plants (Fig. 3) were transplanted into the substrate and acclimatised in the laboratory (common growth chamber) and in the greenhouse (moist chamber). The acclimatisation in the moist chamber was found to be more efficient (90.9% of plants survived). In the common growth chamber, the percentage was obviously lower (68.1% of plants survived).

Fig.3: Roots obtained on the rooting medium R_1 (A) and plantlets after acclimatisation (B) (Gošnjak 2009)

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