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IN VITRO PROPAGATION OF SOME ROSE CULTIVARS

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Abstract. The paper presents aspects regarding the response of 5 rose cultivars from the collection of rose cultivars of the Fruit Research Station of Cluj in the process of micropropagation (multiplication, rooting, and acclimation). The cultivars studied were: Simina, Rusticana, Pasiune, Magic HT and The Fairy. In order to establish the differences between the 5 cultivars regarding multiplication rate, the Murashige & Skoog (MS) medium (1962) was used + 0.7 mg/l BAP; in order to establish the rooting percentages hormone-free MS medium was used and for acclimation hydroculture was used. Multiplication rates varied with the cultivar, Pasiune having the highest multiplication rate.

Key words: micropropagation, multiplication rate, rooting, hydroculture.

INTRODUCTION

Its colouring generosity and its diversity of growth types (shrub, climber, ground cover, dwarf, miniature) situates roses among the most widespread ornamental plants for parks, gardens, terraces, that's why research regarding the breeding of new cultivars as well as improving propagation technologies is in continuous expansion. Due to its advantages, micro-propagation was experimented by many researchers for this species also (Al-Khalifah et al., 2005, Hameed et al. 2006, Khosh-Khui and Jabbarzadeh 2007, Maior et al. 2007, Ozel and Arslan 2006, Senapati and Rout 2008). As basal media, diverse variants of MS were used and, as growth regulators, BAP, Kinetin and TDZ were used. Having in view the tradition of the Fruit Research station of Cluj for the breeding of rose cultivars (40 bred and homologated cultivars), in the framework of the Plant tissue culture laboratory the micro-propagation of newly created cultivars as well as other cultivars in the collection was also researched.

MATERIALS AND METHODS

The rose cultivars Simina (1996, author St. Wagner), Rusticana (2000, author St. Wagner), Pasiune Mov (authors Gabriela Roman and St. Wagner), Magic Hit and The Fairy (foreign cultivars present in the collection of roses) were studied.

The plant material used for establishing multiplication rate, rooting

percentage and acclimation percentage originated from *in vitro* cultures initiated in the framework of the plant tissue culture laboratory at the Fruit Research Station Cluj. The research carried out in the framework of the laboratory (Clapa and Fira, 2008) has shown that the optimal culture medium for rose *in vitro* culture initiation as well as multiplication was the Murashige & Skoog (MS) medium (1962) slightly modified: MS+0,7 mg/l BAP, whereas for rooting, hormone-free medium proved to be optimal (Table 1).

In order to establish the multiplication rates, the 5 rose cultivars were inoculated onto multiplication medium (Table 1).

All the components were added before media autoclaving. The pH of the media was adjusted before adding the agar. The medium was dispensed into Magenta GA_7 vessels (approx. 50 ml/vessel), 9 explants consisting of 1-1.5 cm long shoot fragments (with an average of approx. 2 nodes/explants), which proved to be the optimal explants' size in the process of rose *in vitro* propagation were inoculated into each vessel. Incubation was done in controlled environmental conditions, providing temperatures of 23-25 °C and light intensity of 2500-3000 luces, with 16-hour photoperiod.

Table 1

Used variants of media for multiplication and rooting

Components	**Initiation/multiplication**	**Rooting**
Microelements	MS*	MS
Macroelements	MS	MS
Vitamins	MS	MS
BAP	0.7 mg/l	-
Vitamin B1	1 mg/l	1 mg/l
Sugar	30 g/l	30 g/l
Plant-Agar	5 g/l	5 g/l
FeNa EDTA	36.7 mg/l	36.7 mg/l
pH	5.8	5.8

* Murashige & Skoog

Establishing multiplication rate was done after 8 weeks in culture, when the newly formed plantlets were transplanted to a hormone-free medium (Table 1) for rooting. After 4-6 weeks on the rooting medium, according to the variety, rooting percentages were calculated. Then the rooted plantlets were transplanted for acclimation into plastic trays containing perlite as well as in hydroculture.

RESULTS AND DISCUSSIONS

In order to establish propagation rates, for each of the 5 cultivars 3 Magenta vessels, each containing 9 plantlets, were taken randomly. The average numbers of inoculi resulting/vessel during subculture were: 91 for Pasiune, 45.2 for cultivar Rusticana, 55.4 for cultivar Simina, 77 for cultivar The Fairy and 59.3 for Magic HT. The multiplication rates, respectively the number of plantlets resulted per vitro-plant per multiplication cycle for the 5 cultivars were 10.1 for cultivar Pasiune, 5.02 for cultivar Rusticana, 6.07 for cultivar Simina, 6.6 for cultivar Magic HT and 8.5 for cultivar The Fairy (Fig. 1).

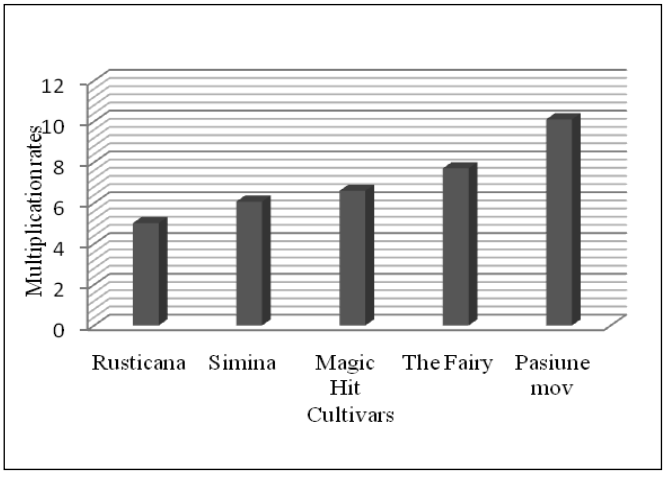

Fig. 1. Multiplication rates

The results indicate that multiplication rates vary with the cultivar, the highest multiplication rate being obtained for cultivar Pasiune, 10.1. This cultivar presented vigorous, well-differentiated, uniform shoots (Fig. 2, 3). In cultivar Rusticana the shoots were non-uniform, from one plantlet 1-2 long, vigorous shoots were obtained, 4-6 cm in length and the others were short, 3-4 cm in length (Fig. 4). In cultivar The Fairy reddish, non-uniform shoots were obtained, presenting rich foliage, the basal leaves died much sooner than in the other cultivars and the vitroplants rooted and bloomed on the multiplication medium (Fig. 5). Cultivars Magic HT and Simina generated a rich mass of uniform, well-differentiated shoots which were shorter than in the preceding cultivars.

Fig. 2. Cultivar Pasiune – multiplication phase

Fig. 3. Cultivar Pasiune – *in vitro* proliferation

Fig.4. Cultivar Rusticana- multiplication phase

Fig. 5. Cultivar The Fairy – multiplication phase

The shoots obtained on MS medium + 0.7 mg/l BAP were transferred to hormone-free MS medium (Table 1) in order to monitor their reaction and rooting. The shoots started to form roots 2-3 weeks after transplanting to rooting medium and the plantlets became suitable for *ex-vitro* transfer for acclimation in 4-6 weeks varying with the cultivar. In cultivars Pasiune and The Fairy the plantlets could be acclimated after 4 weeks of culture in the rooting medium and the other cultivars could be acclimated after 6 weeks. In all the cultivars, vigorous, well-developed plantlets were obtained, with well-developed roots (Fig. 6).

Fig. 6. *In vitro* rooting on hormone-free MS medium

The rooting percentages are presented in Table 2, the highest rooting percentage being obtained for cultivar The Fairy, 97,21%.

Table 2

Rooting percentages

Cultivars	Total no. of tested plants	No. of rooted plants	Rooting percentages (%)
Pasiune mov	64	52	81,25
Simina	99	89	89,89
Rusticana	94	87	92,55
The Fairy	74	72	97,29

In the first phase, in cultivars Rusticana and Simina acclimation was done in perlite, in covered plastic trays and the plantlets got acclimated in about 4 weeks.

Because this method needs special conditions of temperature and humidity, which are rather costly and difficult to provide, an alternative method was used instead, acclimation in hydroculture. This method consists in the culture of rose plants that had been well-rooted *in vitro*, in uncovered plastic or glass vessels in which a layer of water having neutral pH was poured, 1.5-2 cm thick, just to cover the roots. This method provides atmospheric humidity and ventilation needed for the plantlets during *ex-vitro* acclimation. The method of acclimation in hydroculture was successfully applied for all the cultivars taken into study (Fig. 7 and 8). The results obtained showed that this method was more effective and less costly than traditional acclimation in perlite.

Fig. 7. Acclimation in hydroculture

Fig. 8. Plants acclimated in hydroculture

It is important that the acclimated plants must be planted into pots into potting mix after 4-5 weeks of acclimation; otherwise there is the danger for the plantlets to rot. The acclimated plantlets were planted into pots, self-rooted plants being obtained (Fig. 9).

Fig. 9. Self-rooted rose plants obtained *in vitro*

CONCLUSIONS

The culture medium MS + 0.7 mg/l BAP proved to be efficient in all the rose cultivars taken into study, providing good multiplication rates and at the same time the regeneration of vigorous plantlets in all the 5 cultivars.

8 weeks after inoculation the shoots resulted on the variants containing 0.7 mg/l BAP can be transferred to hormone-free medium for rooting. After 4-6 weeks, varying with the cultivar, rooted plantlets are obtained which can be acclimated *ex-vitro* in perlite or in hydroculture.

Acclimation in hydroculture presents the advantage that it necessitates a small amount of space in the greenhouse or growth chamber and necessitates a lower amount of labour as compared to acclimation in perlite.

In vitro micropropagation is an efficient way of propagating roses, by which self-rooted rose plants can be obtained, eliminating the need for grafting.

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