Ornamental Horticulture
ISSN 2447-536X | WWW.ORNAMENTALHORTICULTURE.COM.BR
SBFPO Sociedade Brasileira de Floricultura e Plantas Ornamentais

ARTICLE

Features of the clonal micropropagation technology of ornamental Rose varieties 'Dream Come True' and 'Full Sail'

Otimização da tecnologia de micropropagação clonal de variedades ornamentais de rosas 'Dream Come True' e 'Full Sail'

Sergey Sergeevich Makarov[1*], Irina Borisovna Kuznetsova[2], Muthab Hussien[3], Anton Igorevich Chudetsky[1] and Anastasia Ivanovna Sokolkina[1]

[1] Russian State Agrarian University - Moscow Timiryazev Agricultural Academy, Department of Ornamental Horticulture and Lawn Science, Department of Landscape Architecture and Artificial Forests, Moscow, Russian Federation.
[2] Kostroma State Agricultural Academy, Department of Agrochemistry, Biology and Plant Protection, Kostroma, Russian Federation.
[3] Tsitsin Main Botanical Garden of the Russian Academy of Sciences, Moscow, Russian Federation.

Abstract: The rose is the most important ornamental and floral crop, as well as a valuable aromatic and medicinal plant. For accelerated production of planting material, clonal micropropagation is used. There are problems in obtaining an aseptic culture and the peculiarities of plant cultivation at different stages, depending on the variety. It is necessary to develop and optimize the technological cycle of cultivation in *in vitro* culture. The results showed that the highest viability of rose explants obtained from lateral buds was noted by sterilization with 0.2% silver nitrate solution of and 5% Lysoformin 3000 with an exposure of 15 minutes (82%-95%); the highest viability of explants obtained from etiolated shoots was observed when sterilized with 0.2% silver nitrate solution with an exposure of 10 minutes and 5% Lysoformin 3000 with an exposure of 15 min (82%-100%). The maximum length of the micro-shoots was noted in the 'Dream Come True' variety on a QL (Quoirin and Lepoivre) nutrient medium (on average 21.2 cm), in the 'Full Sail' variety – on a ½ QL medium (on average 24.9 cm). An increase in the concentration of 6-BAP from 0.5 to 1.0 mg L^{-1} in the nutrient medium contributed to an increase in the number of micro-shoots (by 1.7–1.9 times). The maximum root length was noted on the nutrient medium ½ QL (on average 88.7–95.3 cm). An increase in the concentration of IAA from 0.5 to 1.0 mg L^{-1} in the nutrient medium contributed to an increase in root length by 1.4–1.8 times. The protocol developed in this study allows to propagate these two varieties *in vitro* and produce a large number of plants.
Keywords: growth regulators, *in vitro,* nutrient medium, rosa.

Resumo: A rosa é a cultura ornamental mais importante, além de uma valiosa planta aromática e medicinal. Para produção acelerada de grande quantidade de material de plantio, utiliza-se a micropropagação clonal. Porém, existem problemas na obtenção de uma cultura asséptica e nas peculiaridades do cultivo das plantas nas diferentes fases, dependendo da variedade. Para algumas variedades de rosas é necessário desenvolver e otimizar o ciclo tecnológico de cultivo *in vitro*. Os resultados mostraram que a maior viabilidade dos explantes de rosa obtidos das gemas laterais foi observada com esterilização com soluções de nitrato de prata a 0,2% e Lysoformin 3000 a 5% e exposição de 15 minutos (82%-95%); a maior viabilidade dos explantes obtidos de brotos estiolados foi observada quando esterilizados com soluções de nitrato de prata 0,2% com exposição de 10 minutos e Lysoformin 3000 5% com exposição de 15 min (82%-100%). O comprimento máximo dos microbrotos de rosa foi observado na variedade 'Dream Come True' em meio nutriente QL (em média 21,2 cm), na variedade 'Full Sail' – em meio ½ QL (em média 24,9 cm). Um aumento na concentração de 6-BAP de 0,5 para 1,0 mg L^{-1} no meio de cultivo contribuiu para um aumento no número de brotações (de 1,7 a 1,9 vezes). O comprimento máximo da raiz das variedades estudadas foi observado no meio de cultivo½ QL (em média 88,7–95,3 cm). Um aumento na concentração de AIA de 0,5 para 1,0 mg L^{-1} no meio de cultivo contribuiu para um aumento no comprimento da raiz em 1,4–1,8 vezes. O protocolo desenvolvido neste estudo permite propagar essas duas variedades in vitro e produzir um grande número de plantas.
Palavras-chave: reguladores de crescimento, *in vitro*, meio de cultivo, rosa.

Introduction

The rose has long been known not only as an important ornamental and floral crop, but also as a valuable aromatic and medicinal plant. In recent decades, not only many new varieties of roses have appeared, but also individual garden groups with their own unique characteristics (Datta, 2018; Churikova and Krinitsyna, 2020; Bernardis et al., 2022). All this leads to an increased demand for planting material of the rose culture in the modern world market at the present time. An urgent task is to accelerate the production of a large number of seedlings, especially valuable, rare or difficult-to-propagate varieties.

Many years of practical experience have shown that cuttings as a traditional method of roses propagation is very laborious and not always effective, whereas most cultivated varieties are not native. At the same time, the limiting factors in obtaining planting material for roses are also the low rate of reproduction and the dependence of plants on the season (Alderson, 1988; Kentelky et al., 2023). In order to obtain the required number of seedlings of valuable rose varieties, it is necessary to resort to the use of biotechnological methods, such as clonal micropropagation, which allows you to obtain a large amount of high-quality, genetically homogeneous and healthy root-related planting material from viral and fungal infection in a short period of time throughout the year (Butenko, 1999; Kaushal et al., 2023). At the same time, today certain varieties of roses are sparsely distributed and difficult to propagate, and its cultivation *in vitro* conditions will contribute to solving this problem, as well as preserving the gene pool of valuable plants, maintaining the biodiversity of botanical collections and providing planting material for nurseries, garden centers, and private entrepreneurs.

The results of numerous studies on the clonal micropropagation of representatives of the genus *Rosa* L. and micro-shoots its hybrids, conducted by scientists from all over the world from the middle of the XX century to the present day, still show both the problems of obtaining an aseptic culture and the peculiarities of growth and development of regenerating plants at different stages of microcloning, depending on the species and variety (Badzian et al., 1991; Carelli and Echeverrigaray, 2002; Pati et al., 2006; Attia et al., 2012; Khosh-Khui, 2014; Liu et al., 2018; Aggarwal et al., 2020; Churikova and Krinitsina, 2020; Matos et al., 2021; Samiei et al., 2021; Afrin et al., 2022; Harmon et al., 2022; Al-Ali et al., 2023; Al-Rekaby, 2023; Assadi-Sheikh et al., 2023; Rezanejad et al., 2023; Seyed Hajizadeh and Azizi, 2023; Rahman et al., 2023; Şirin et al., 2023). There are also difficulties with the rooting of plants obtained *in vitro* at the stage of their adaptation to non-sterile conditions (Kuznetsova and Egorova, 2018; Vasilyeva et al., 2022).

In this regard, it is necessary to develop and optimize the full technological cycle of rose cultivation in vitro, and for some varieties of roses, additional research is necessary. So, the aim of this research is to investigate the characteristics of clonal micropropagation for the rose varieties 'Dream Come True' and 'Full Sail' during the stages of introduction, micropropagation, and in vitro rooting of microshoots.

***Corresponding author:** s.makarov@rgau-msha.ru | https://doi.org/10.1590/2447-536X.v30.e242732 | Editor: Bruno Trevenzoli Favero, University of Copenhagen, Dinamarca | Received: Mar 03, 2024 | Accepted: July 29, 2024 | Available online: Aug 15, 2024 |

Materials and Methods

Plant material

The research was conducted in the Educational and Scientific Laboratory of Microclonal Plant Reproduction and Experimental Hydroponics at the Russian State Agrarian University – Moscow Timiryazev Agricultural Academy using generally accepted methods (Butenko, 1999) in 2023-2024. The objects of the study were plants of cultivated roses of the ornamental varieties 'Dream Come True' and 'Full Sail'. Explants obtained from the lateral buds and etiolated plant shoots from cuttings harvested in late April were used as the initial plant material.

Sterilization of explants

Various sterilizing solutions were used to sterilize the plant material: 5% sodium hypochlorite, 10% hydrogen peroxide, 0.2% mercuric dichloride, 0.2% silver nitrate ($AgNO_3$), as well as modern disinfectants – 0.01% Nika-2 (Genix, Russia) and 5% Lysoformin 3000 (Gigiena Plus, Russia). Explants were planted for each variant of the experiment in the amount of 100 pieces. Exposure time is 3, 5, 10, and 15 minutes. The plants were cultivated on a modified nutrient medium according to the Quoirin and Lepoivre (QL) (1977) recipe in variants of diluting the mineral base with bidistilled water by 2 and 4 times (the pH level of the medium is 5.6-5.8). At the stage of *in vitro* culture introduction, the number of viable explants (%) was taken into account.

Further, the cultivation of regenerating plants was carried out using the same nutrient medium in a light room at a 16-hour photoperiod, air temperature +23-+25 ° C, air humidity 75%-80%.

In vitro shoot formation and microcutting

At the stage of microgrowth proliferation, 6-benzylaminopuryl (6-BAP) as a cytokinin was added to the QL nutrient medium at concentrations of 0.5 and 1.0 mg L^{-1}. Biometric indicators (the number and length of micro-shoots) were taken into account per regenerating plant. The repetition of the experiment is 3-fold, with 10 plants in each.

In vitro rooting of micro-shoots

At the stage of root formation, indolylacetic acid (IAA) as an auxin was added to the QL nutrient medium at concentrations of 0.5 and 1.0 mg L^{-1}. Biometric indicators (the number and length of roots) were taken into account per regenerating plant. The repetition of the experiment is 3-fold, with 10 plants in each.

Statistical data processing

Statistical processing of the experimental data for different stages of micropropagation obtained was carried out according to generally accepted methods (Dospekhov, 2011) using Microsoft Office Excel 2019 and StatSoft Statistica v13.3 software tools. The reliability of differences between the average data of the experimental variants was assessed using three-factor analysis of variance and the least significant difference for 5% of the significance level (LSD_{05}).

Results and Discussion

Sterilization of explants

At the stage of *in vitro* introduction of the rose varieties 'Dream Come True' and 'Full Sail' into culture, we studied the effect of the main sterilizers and the time of their exposure on the viability of explants from lateral buds and etiolated shoots. In explants from lateral buds, the highest viability was observed when sterilized with 0.2% silver nitrate (90%-95%) and 5% Lysoformin 3000 (82%-87%) with an exposure of 15 minutes. When using the same sterilizing solutions with an exposure of 10 minutes, the explant viability of the 'Dream Come True' variety was 85% and 72%, and that of the 'Full Sail' variety was only 64% and 62%, respectively. Sterilization of explants from lateral buds for 3 and 5 minutes turned out to be clearly insufficient, since less than 14%-16% and 33%-35% of specimens, respectively, turned out to be viable in this case (Table 1).

Table 1. The viability of the rose explants (n = 200) depends on the sterilizing agent and the exposure time, %

Variety	Sterilizing agent	Exposure time, min			
		3	5	10	15
Explants from the lateral buds					
'Dream Come True'	Sodium hypochlorite 5%	8	24	50	60
	Hydrogen peroxide 10%	12	25	51	42
	Mercuric dichloride 0.2%	10	35	60	38
	$AgNO_3$ 0.2%	14	17	85	95
	Nika-2 0.01%	12	26	70	46
	Lysoformin 3000 5%	6	32	72	82
'Full Sail'	Sodium hypochlorite 5%	12	34	48	56
	Hydrogen peroxide 10%	4	29	59	38
	Mercuric dichloride 0.2%	3	31	61	42
	$AgNO_3$ 0.2%	10	26	64	90
	Nika-2 0.01%	11	30	70	78
	Lysoformin 3000 5%	16	33	62	87
Etiolated shoots					
'Dream Come True'	Sodium hypochlorite 5%	20	30	70	65
	Hydrogen peroxide 10%	28	42	72	60
	Mercuric dichloride 0.2%	24	46	67	52
	$AgNO_3$ 0.2%	10	40	98	77
	Nika-2 0.01%	22	24	70	64
	Lysoformin 3000 5%	22	44	80	82
'Full Sail'	Sodium hypochlorite 5%	10	40	70	58
	Hydrogen peroxide 10%	8	52	71	50
	Mercuric dichloride 0.2%	20	54	70	46
	$AgNO_3$ 0.2%	24	38	100	74
	Nika-2 0.01%	16	26	72	62
	Lysoformin 3000 5%	12	34	76	92

In explants from etiolated shoots, the maximum viability was noted when using 0.2% $AgNO_3$ with an exposure of 10 minutes (98%-100%), and with an increase in processing time to 15 minutes, the viability decreased to 77-74%. In variants with 5% Lysoformin 3000, 82-92% of explants were viable at an exposure of 15 minutes, and 76%-80% at an exposure of 10 minutes.

In various research protocols for the sterilization of rose explants, the best results were obtained using sterilizing agents such as a 5.00%-5.25% sodium hypochlorite solution for 10 or 20 minutes, achieving viability rates of up to 85% (Carelli and Echeverrigaray, 2002). Our studies demonstrated similarly high viability rates using Lysoformin 3000, which was employed for the first time in rose explant sterilization. Additionally, our positive results using $AgNO_3$ as a sterilizing agent align with other studies, where rose explant viability reached 98% (Matos et al., 2021).

It should be noted that in this type of explants, when sterilized with 5% sodium hypochlorite, 10% hydrogen peroxide, 0.2% mercuric dichloride and 0.01% Nika-2 for 10 minutes, the viability varied between 67%-72%. Treatment for 3 and 5 minutes was ineffective, 46%-90% of explants died from infection. Under similar sterilization conditions, the viability of explants of the studied rose varieties from etiolated shoots was higher than from lateral buds (Table 1).

***In vitro* shoot formation and microcutting**

In the course of research at the stage of micro-shoots proliferation, we found that the average number of rose micro-shoots was maximum on the nutrient medium of ½ QL, which recorded 11.7 pcs in the 'Dream Come True' variety, 11.3 pcs in the 'Full Sail' variety. At the same time, this indicator was 1.3–1.6 times lower on the QL medium, 1.4–1.6 times lower on the QL medium. An increase in the concentration of cytokinin 6-BAP from 0.5 to 1.0 mg L^{-1} in the nutrient medium contributed to an increase in the number of micro–shoots of roses of the 'Dream Come True' variety by 1.9 times, of the 'Full Sail' variety by 1.7 times (Table 2).

Table 2 The number of micro-shoots of roses *in vitro*, depending on the composition of the nutrient medium and the concentration of 6-BAP, pcs.

Nutrient medium compositon	Concentration of 6-BAP, mg L^{-1}		Mean
	0.5	1.0	
'Dream Come True'			
QL	5.9±0.44	10.9±0.88	8.4
½ QL	7.1±0.60	16.3±1.12	11.7
¼ QL	6.9±0.54	10.1±0.84	8.5
Medium	6.6	12.4	-
LSD_{05}: A = 1.32, B = 1.42, AB = 1.83			
'Full Sail'			
QL	5.1±0.40	8.7±0.72	6.9
½ QL	7.7±0.64	14.9±1.30	11.3
¼ QL	6.0±0.52	8.1±9.70	7.1
Medium	6.3	10.6	-
LSD_{05}: A = 1.40, B = 1.21, AB = 2.01			

The average length of the rose micro-shoots was greatest on the QL nutrient medium and it averaged 2.7 cm for the 'Dream Come True' variety, 2.3 cm for the 'Full Sail' variety, whereas 1.6 and 2.2 cm for the ½ QL medium, respectively, and 1.1 and 1.2 cm for the ¼ QL medium. An increase in the concentration of 6-BAP from 0.5 to 1.0 mg L^{-1} in the nutrient medium did not significantly affect the average length of micro-shoots of roses of the studied varieties (Table 3).

Table 3. The average length of rose micro-shoots *in vitro*, depending on the composition of the nutrient medium and the concentration of 6-BAP, cm

Nutrient medium	Concentration of 6-BAP, mg L^{-1}		Mean
	0.5	0.5	
'Dream Come True'			
QL	3.3±0.25	2.1±0.17	2.7
½ QL	1.5±0.10	1.6±0.13	1.6
¼ QL	1.0±0.06	1.2±0.09	1.1
Medium	1.9	1.6	-
LSD_{05}: A = 0.89, B = 0.72, AB = 1.01			
'Full Sail'			
QL	2.5±0.21	2.0±0.18	2.3
½ QL	3.0±0.26	1.3±0.11	2.2
¼ QL	1.3±0.10	1.0±0.08	1.2
Medium	2.3	1.4	-
LSD_{05}: A = 1.03, B = 1.17, AB = 1.49			

The total length of the micro-shoots of the rose 'Dream Come True' variety was significantly longer on the QL nutrient medium and averaged 21.2 cm, while on the ½ QL medium it was 1.1 times less, on the ¼ QL medium it was 2.3 times less. The 'Full Sail' variety had the largest total length on the medium ½ QL, it reached 24.9 cm, on the medium QL it was 1.6 times less, on the medium ¼ QL – 2.9 times.

With an increase in the concentration of cytokinin 6-BAP in the nutrient medium from 0.5 to 1.0 mg L^{-1}, the total length of micro-shoots of roses increased significantly in the 'Dream Come True' variety by 1.6 times, whereas no significant differences were found for the 'Full Sail' variety (Table 4).

Table 4. The total length of micro-shoots of roses *in vitro*, depending on the composition of the nutrient medium and the concentration of 6-BAP, cm

Nutrient medium	Concentration of 6-BAP, mg L^{-1}		Mean
	0.5	1.0	
'Dream Come True'			
QL	19.5±1.75	22.9±2.14	21.2
½ QL	10.7±0.98	26.1±2.49	18.7
¼ QL	6.9±0.62	12.2±1.14	9.4
Medium	12.4	20.4	-
LSD_{05}: A = 0.84, B = 0.92, AB = 1.16			
'Full Sail'			
QL	12.8±0.10	18.3±0.14	15.9
½ QL	23.1±0.22	19.4±0.15	24.9
¼ QL	7.8±0.06	8.1±0.07	8.5
Medium	14.6	15.3	-
LSD_{05}: A = 0.78, B = 0.62, AB = 0.98			

The maximum reproduction coefficient (C_m = 8) in the rose of the 'Dream Come True' variety was detected on the QL nutrient medium on the 3rd and 5th passages, in the 'Full Sail' variety – on the ½ QL medium on the 6th and 7th passages. Starting from the 7th passage, a moderate decrease in the reproduction coefficient was observed in all the studied varieties. Therefore, it is advisable to microcutting the studied rose varieties up to and including the 7th passage, when the plants have the greatest regenerative ability (Fig. 1).

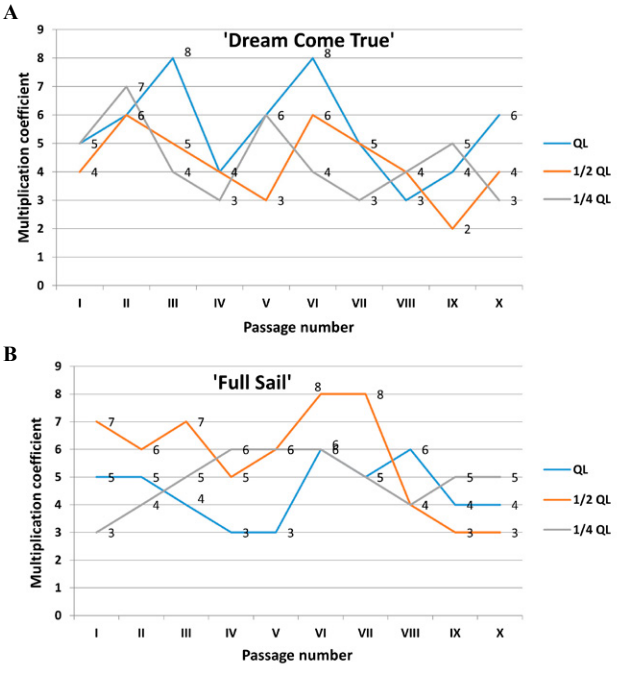

Fig. 1. The multiplication coefficient of rose varieties 'Dream Come True' (A) and 'Full Sail' (B) on the nutrient medium QL in various modifications depending on the number of passages.

In other protocols for clonal micropropagation of roses at the in vitro shoot formation and microcutting stage, good results were primarily observed using the Murashige and Skoog (MS) nutrient medium (Badzian et al., 1991; Attia et al., 2012; Liu et al., 2018; Aggarwal et al., 2020; Afrin et al., 2023; Al-Ali et al., 2023). However, a few studies using the QL nutrient medium for in vitro propagation of roses have also shown its effectiveness. For example, the addition of 3.0 mg L^{-1} 6-BAP and 0.5 mg L^{-1} NAA yielded good results for the varieties 'Khosh-Khui' and 'Sink' (Carelli and Echeverrigaray, 2002).

Our results consistent with the results of other studies indicating that 6-BAP at a concentration of 2.0–3.0 mg L^{-1} positively affects the proliferation and elongation of hybrid rose shoots, even compared to other plant growth regulators such as kinetin and 2-iP (Aggarwal et al., 2020; Carelli and Echeverrigaray, 2002; Attia et al., 2012; Al-Ali et al., 2023; Rahman et al., 2023).

In vitro rooting of micro-shoots

At the stage of root formation in *in vitro* culture, a significantly larger number of rose roots were formed on the nutrient medium ½ QL (on average 6.2–6.5 pcs.), whereas on the medium QL – less by 1.4 times, on the medium ¼ QL – by 2.0–2.1 times. There were no significant differences in the number of roots of the studied rose varieties depending on the concentration of auxin IAA (Table 5).

The average length of the rose roots was maximum on the medium ½ QL - on average 14.2–14.5 cm, while on the medium QL – 5.6–6.1 cm, on the medium ¼ QL – 5.0–5.7 cm. With an increase in the concentration of auxin IAA from 0.5 to 1.0 mg L^{-1} in the nutrient medium, the average root length of the studied varieties increased by 1.2–1.3 times (Table 6).

The average length of the rose roots was maximum on the nutrient medium of ½ QL and was 95.3 cm on average for the 'Dream Come True' variety, 88.7 cm for the 'Full Sail' variety, which is significantly more than for QL (3.6 and 3.2 times, respectively) and ¼ MS (6.3 and 5.1 times). With an increase in the concentration of auxin IAA from 0.5 to 1.0 mg L^{-1} in the nutrient medium, the total length of the rose roots of the studied varieties increased by 1.4–1.8 times (Table 7).

Table 5. The number of rose roots, depending on the composition of the nutrient medium and the concentration of IAA, pcs.

Nutrient medium	IAA concentration, mg L^{-1}		Mean
	0.5	1.0	
'Dream Come True'			
QL	4.1±0.34	5.2±0.04	4.6
½ QL	5.3±0.45	7.6±0.06	6.5
¼ QL	2.9±0.25	3.2±0.28	3.1
Medium	4.1	5.3	-
LSD_{05}: A = 1.10, B = 1.32, AB = 1.69			
'Full Sail'			
QL	3.8±0.32	4.9±0.40	4.4
½ QL	5.5±0.44	6.9±0.61	6.2
¼ QL	3.2±0.29	3.0±0.25	3.1
Medium	4.2	4.9	-
LSD_{05}: A =1.03, B =1.16, AB = 1.54			

Table 6. The average length of the rose roots, depending on the composition of the nutrient medium and the concentration of IAA, cm

Nutrient medium	IAA concentration, mg L^{-1}		Mean
	0.5	1.0	
'Dream Come True'			
QL	4.3±0.35	6.9±0.60	5.6
½ QL	12.6±1.05	16.3±1.44	14.5
¼ QL	4.6±0.38	5.3±0.46	5.0
Medium	7.2	9.5	-
LSD_{05}: A =1.36, B = 1.59, AB = 2.46			
'Full Sail'			
QL	5.1±0.45	7.1±0.62	6.1
½ QL	13.2±1.28	15.2±1.45	14.2
¼ QL	5.2±0.46	6.1±0.52	5.7
Medium	7.8	9.5	-
LSD_{05}: A =1.32, B =1.53, AB = 2.50			

Table 7. The total length of the rose roots, depending on the composition of the nutrient medium and the concentration of IAA, cm

Nutrient medium	IAA concentration, mg L^{-1}		Mean
	0.5	1.0	
'Dream Come True'			
QL	17.6±1.54	35.9±0.31	26.8
½ QL	66.8±0.57	123.8±10.52	95.3
¼ QL	13.3±1.06	17.0±0.14	15.2
Medium	32.6	58.9	-
LSD_{05}: A = 2.10, B = 2.68, AB = 3.02			
'Full Sail'			
QL	19.4±1.80	34.8±3.14	27.1
½ QL	72.6±0.63	104.9±9.86	88.7
¼ QL	16.6±0.12	18.3±0.15	17.5
Medium	36.2	52.6	-
LSD_{05}: A =1.99, B =2.71, AB = 3.21			

The use of IBA at the rooting stage of rose microshoots has produced the most positive results (Attia et al., 2012; Aggarwal et al., 2020; Afrin et al., 2022; Rahman et al., 2023). For example, optimal root induction of Rosa sp. was achieved using ½ MS nutrient medium supplemented with 1.0 mg L^{-1} IBA and 1.0 mg L^{-1} NAA (Afrin et al., 2022). Similarly, for rooting micro-shoots of *Rosa* × *hybrida* L. cv. 'Al-Taif Rose,' the best results were observed on MS nutrient medium supplemented with 2.0 mg L^{-1} IBA (Attia et al., 2012). Our results for the number and length of roots of regenerated rose plants in vitro using IAA exceed those reported in other studies (Carelli and Echeverrigaray, 2002) and are comparable to those using IBA. This may be attributed to the use of a different nutrient medium (QL instead of MS) and the specific varietal characteristics.

The results obtained confirm the success of using proven nutrient compositions and growth regulators in micropropagation of both other varieties of roses and some berry crops with high ornamental qualities (during flowering, fruiting and leaf color changes) (Makarov et al., 2021a; 2021b; 2023).

Conclusions

In this work, for the first time, elements of the technology of clonal micropropagation for rose varieties 'Dream Come True' and 'Full Sail' were developed. The highest viability of etiolated shoot could be obtained by using 0.2% silver nitrate and 5% Lysoformin 3000. The ½ QL nutrient medium containing1.0 mg L^{-1} 6-BAP increased both the number and length of rose micro-shoots. At the stage of rooting the ½ QL medium supplemented with 1.0 mg L^{-1} IAA led to better rooting rose micro-shoots.

Acknowledgements

The article was made with support of the Ministry of Science and Higher Education of the Russian Federation in accordance with agreement № 075-15-2022-317 date April 20, 2022 on providing a grant in the form of subsidies from the Federal budget of Russian Federation. The grant was provided for state support for the creation and development of a World-class Scientific Center "Agrotechnologies for the Future".

Author Contribution

SM: Conceptualization; Methodology; Supervision; Writing – Review & Editing; Project Administration. **IK:** Investigation; Visualization; Writing – Original Draft. **MH:** Data Curation; Software; Writing – Review & Editing. **AC:** Formal Analysis; Methodology; Writing – Original Draft. **AS:** Investigation; Validation; Resources.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data will be available on request.

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