

# Biotechnology as a heart of innovation in nursery management of ornamental plants

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**Abstract**—The article presents the results of three-year studies on microclonal propagation of climbing roses «PalaisRoyal», «Camelot» and «Nahema». At the stage of proliferation the optimum concentration of cytokinin 6-benzylaminopurine (6-BAP) in the Murashige and Skoog medium (MSO) for three varieties of roses was 1.0 mg/L. With an increase in the cytokinin content to 2.0 mg/l, a tendency to the proliferation coefficient increase was observed. Positive average correlation dependence of the proliferation coefficient on the size of a cutting was revealed. The use of the commercial product "Siliplant" as trace elements had a positive effect on lengthening of the microcuttings of three rose varieties. The proliferation coefficient was not significantly different from the control one. In order to prepare for the rhizogenesis stage, glucose is the best carbohydrate in the composition of the nutrient medium compared to sucrose, as evidenced by a statistically valid improvement in the quality of the cuttings. The optimal cultivation soil for the adaptation of rose seedlings is a physico-chemical-balanced soil mixture based on highmoor and transitional peat.

**Keywords**—*microclonal propagation, climbing roses, growing medium, hormones, adaptation, substrate.*

## I. INTRODUCTION

The climbing roses are one of the leading causes in vertical gardening of gazebos, walls and buildings, perfectly combining with architectural forms of large and small sizes. They are indispensable for creating such decorative garden structures as pyramids, columns, garlands, gazebos and arches. Climbing roses more often propagated by layering, but this method gives a slight yield of planting material [1].

The complexity of vegetative propagation led to the search for more effective methods of rose multiplication. Microclonal propagation *in vitro* is an alternative approach, thanks to which you can get the maximum amount of virus-free, good quality planting material of the original genotype within a relatively short period of time [2]. The ability to work throughout the year and saving space required for the cultivation of planting material also counts as an advantage. Using reproduction *in vitro* technology up to 400,000 plants can be cloned from a single rose bush per year [3].

The biotechnological method of plants propagation has many advantages in comparison with traditional vegetative methods: high rate of reproduction, planting material is free from various kinds of pathogens and passes through rejuvenation stage, miniaturization of all stages, and release

of material by set term, eliminating the need for keeping grafters and fog cannon, etc.

Work objective: stage optimization of microclonal propagation of climbing roses «PalaisRoyal», «Camelot», «Nahema».

## II. MATERIALS AND RESEARCH TECHNIQUES

The objects of research were micro cuttings and micro seedlings of climbing roses of PalaisRoyal (Meilland France, 2005), Camelot (Tantau Germany, 2011), Nahema (Delbard France, 1999) varieties.

The studies were conducted on the base of biotechnological laboratory of the department of plant introduction and plant establishment of Udmurt Federal Research Center of the Ural Branch of the Russian Academy of Sciences. For introduction into *in vitro* sample shoot apices in the period of maximum growth were used. In the laboratory conditions, all leaf blades were removed and the shoots were cut into 1-2 gemmaceous cuttings. Within 30 minutes, the cuttings were washed under running water in order to remove surface contamination.

The cuttings were sterilized for 8 minutes under the laminar flow cabinets conditions in hydrogen peroxide (33%), followed by 5 times washing in a sterile distillate. The severing cuts on the cuttings were renewed before planting on the nutrient medium.

Micropropagation was carried out on agar Murashige and Skoog medium (MSO) in biological test tubes under light-room conditions at a photoperiod duration of 16 hours and a temperature of  $25 \pm 2$  ° C. In each variant 10 test tubes were taken in triplicate for biometric analysis. The duration of subculturing is 30-35 days. Using generally accepted methods the statistical processing was carried out [4].

At the stage of proliferation, we studied the success of reproduction depending on the concentration of cytokinin 6-BAP, the effect of commercial silicon containing microfertilizers "Siliplant" as trace elements (1 ml / l) and a double concentration of iron (10 mg/l) in the Murashige and Skoog medium.

The proliferation coefficient (pieces /cutting) was calculated as the number of microshoots obtained in one subculturing from a single cutting. The height of micro cuttings was measured using a ruler (mm).

The effect of the composition of the soil substrate and the genotype of roses was investigated while adapting micro seedlings. Micro seedlings were obtained by rooting on Gamborg-Evelega [5] nutrient medium containing 1 mg/l of indolyl-3-acetic acid (IAA). Regenerants with a height no less than 1.5 cm, with white roots in an amount of no less than 3 pieces, with length no less than 0.5-1.0 cm, with the number of leaves no less than 5 pcs were selected.

Different soil substrates were used for adaptation: laboratory soil mixture - lowland peat, vermiculite, biohumus at the ratio 1:0,3:0,3, nitrogen content (NH<sub>4</sub> +NO<sub>3</sub>) – 50 mg/kg, phosphorus (P<sub>2</sub>O<sub>5</sub>) – 200 mg/kg, potassium (K<sub>2</sub>O)– 200 mg/kg, pH5,0 (control); «Torfolin A»: highmoor milled peat, limestone meal, complex mineral fertilizer with macro and microelements, nitrogen content (NH<sub>4</sub> +NO<sub>3</sub>) – 250 mg/kg, phosphorus (P<sub>2</sub>O<sub>5</sub>) – 400 mg/kg, potassium (K<sub>2</sub>O)– 500 mg/kg, pH 6-7, manufacturer - LLC «Fasco»; «Universalnaya sadovaya zemlya» (Universal planting soil): highmoor and transitional peat, biohumus, limestone meal, complex fertilizer, nitrogen content – 250 mg/kg, phosphorus – 300 mg/kg and potassium – 400 mg/kg, pH 5,5, manufacturer - LLC «Ecoprom».

Adaptation was carried out on stagings in micro greenhouses equipped with lamps. Before planting, the substrate was spilled with Trichoderma Veride solution according to the instructions. Micro seedlings were carefully removed from the test tubes with a pair of tweezers; the roots were washed in a decimolar solution of potassium permanganate and planted in a micro greenhouse. In our opinion, washing the roots in potassium permanganate solution from the remnants of an agar nutrient medium delays in part the damage of harmful microorganisms. After planting, the seedlings were sprayed once with “Siliplant” solution (1.5 ml / l) to increase their establishment [6]. Humidity was maintained by daily spraying of a micro greenhouse cover and seedlings by ordinary water. Experiments were performed in 3 replicates. In each variant, at least 60 micro seedlings were analyzed.

III. RESULTS

One of the most important tasks is to obtain the maximum number of microshoots at the stage of proliferation. 6-BAP at a concentration of 1 mg/l was used as cytokinin. The success of proliferation was considered depending on the concentration of 6-BAP in the nutrient medium of 1.0 and 2.0 mg / l (Table 1).

TABLE I. THE PROPAGATION SUCCESS RATE OF ROSES WITH DIFFERENT CONCENTRATIONS OF 6-BAP, PIECES/CUTTING, 2018

Variety, factor A	6-BAP concentration , mg /l		Medium
	1,0	2,0	
PaleRoyal	2,8	4,0	3,4
Camelot	2,9	3,5	3,2
Nahema	3,6	3,7	3,7
Medium	3,1	3,7	
HCP <sub>05</sub>	particular differences		0,9
	phase A main effect		0,6
	phase B main effect		1,1

The proliferation coefficient on the medium with concentration of 6-BAP 1 mg for PaleRoyal, Camelot and Nahema varieties was equal to 2.8, 2.9 and 3.6 pieces / cutting, with an increase in cytokinin medium to 2.0 mg/l - 4.0, 3.5 and 3.7 pieces / cutting, respectively, with HCP<sub>05</sub> =

0.9. On average, for three varieties on medium with a concentration of 6-BAP of 1.0 and 2.0 mg/l, the reproduction success was 3.1 and 3.7 pieces / cutting. No significant differences between the variants of experience revealed (HCP<sub>05</sub>=1,1).

The greatest breeding potential on two growing medium was in the Nahema variety 3.7 pieces / cutting. There was a tendency to reduce the multiplication factor by 0.3 and 0.5 pcs. in PaleRoyal, Camelot varieties (HCP<sub>05</sub>=0,6).

The medium with cytokinin content of 1 mg/l contributed to a significant increase in the microcuttings height as compared with the medium of 2.0 mg/l, at average 31.8 and 29.1 mm respectively (HCP<sub>05</sub>=2,4). The microcuttings height of PaleRoyal variety was 36.7 mm, Camelot and Nahema 28,1 and 26,6 mm respectively in HCP<sub>05</sub>=2,2 (table 2). There is high variety specificity due to the effect of cytokinin concentrations on a particular rose variety. The medium with cytokinin content of 1 mg/l contributed to a significant increase in the microcuttings height as compared with the medium of 2.0 mg/l, at average 31.8 and 29.1 mm respectively (HCP<sub>05</sub>=2,4). The microcuttings height of PaleRoyal variety was 36.7 mm, Camelot and Nahema 28,1 and 26,6 mm respectively in HCP<sub>05</sub>=2,2 (table 2). There is high variety specificity due to the effect of cytokinin concentrations on a particular rose variety.

TABLE II. HEIGHT OF ROSE MICROCUTTINGS DEPENDING ON THE CONCENTRATION OF 6-BAP, MM, 2018

Variety, factor A	6-BAP concentration , mg /l		Medium
	1,0	2,0	
PaleRoyal	38,2	35,2	36,7
Camelot	29,4	26,7	28,1
Nahema	27,8	25,3	26,6
Medium	31,8	29,1	
HCP <sub>05</sub>	particular differences		3,1
	phase A main effect		2,2
	phase B main effect		2,4

The analysis of dependence of the proliferation coefficient on the initial size of the microcutting was carried out. The proliferation coefficient on the medium with concentration of 6-BAP 1 mg for PaleRoyal, Camelot and Nahema varieties was equal to 0.37, 0.39 and 0.45 respectively. This means that there was an average correlation between the signs. A strong correlation was found on the medium with 6-BAP 2.0 mg/l for Camelot variety. The correlation coefficient is 0.75, for the other two varieties it is average.

Thus, the optimal concentration of cytokinin 6-BAP in the composition of the nutrient medium for the PaleRoyal, Camelot and Nahema varieties was 1.0 mg / l. With an increase in cytokinin to 2.0 mg/l, a tendency to increase the proliferation coefficient was observed. A positive average correlation was found between such traits as the size of the cutting and the proliferation coefficient. The exception was Camelot, where the correlation coefficient on the medium with 6-BAP 2.0 mg/l was 0.75.

After the breeding stage not all microcuttings are suitable for transplant for rooting. The completion of growing is necessary for them. Murashige and Skoog medium, cytokinin 6-BAP of low concentration (0.5 mg/l) are usually used. In order to improve the quality of the microcuttings, glucose was included at a concentration of 20 g/l in the composition of the nutrient medium (Fig. 1)

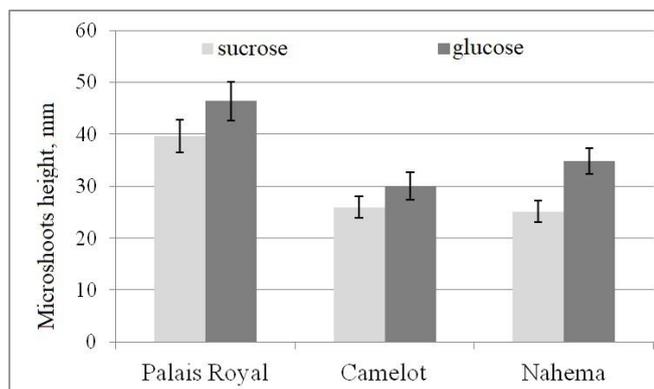


Fig. 1. The effect of sucrose and glucose on the growth and development of roses cuttings, mm, 2018

The microcuttings height on the medium with glucose for all varieties was higher than with sucrose, and the increase was statistically significant ( $p < 0.05$ ).

Thus, to obtain high-quality cuttings for the stage of rhizogenesis on a medium for lengthening, glucose is a better carbohydrate than sucrose.

The analysis of the concentration effect of cytokinin 6-BAP on the proliferation coefficient revealed cultivar-specific reaction of rose cuttings. The proliferation coefficient on the medium with concentration of 6-BAP 1 mg for PaleRoyal, Camelot and Nahema varieties was equal to 2.8, 2.9 and 3.6 pieces / cutting, with an increase in cytokinin medium to 2.0 mg/l - 4.0, 3.5 and 3.7 pieces/cutting, respectively in HCP05=1,9. Thus, an increase in the concentration of cytokinin from 1.0 to 2.0 mg/l led to an increase in the proliferation coefficient depending on the variety in different degree. Thus, PalaisRoyal has the most significant increase in test - by 42.9%. The increase in Camelot and Nahema varieties - by 20.7 and 8.5% respectively. The conclusion about the variety-specific reaction to the conditions of microclonal reproduction is confirmed by the data of other authors. [7].

The greatest potential for reproduction on two growing medium was in the Nahema variety - 3.6 pcs / cutting. There was a tendency to reduce the breeding rate by 0.2 and 0.4 pcs. in PalaisRoyal and Camelot varieties. (HCP05=0,6).

The medium with cytokinin content of 1 mg/l contributed to a significant increase in the microcuttings height compared to the medium of 2.0 mg/l, by an average of 31.8 and 29.1 mm respectively (HCP05=0,6). The microcuttings height of PalaisRoyal variety was 36.7 mm. The microcuttings height of Camelot and Nahema was 28.1 and 26.6 mm in HCP05=2.2.

The analysis of the dependence of the proliferation coefficient on the initial size of the microcutting was carried out. The proliferation coefficient on the medium with concentration of 6-BAP 1 mg for PaleRoyal, Camelot and Nahema varieties was equal to 0.37, 0.39 and 0.45 respectively. This means that there was an average correlation between the signs. A strong correlation dependence was found on the medium with 6-BAP of 2.0 mg/l for Camelot variety, the correlation coefficient is 0.75. For the other two varieties - medium.

Thus, the optimal concentration of cytokinin 6-BAP in the composition of the growing medium for Palais Royal,

Camelot and Nahema varieties was 1.0 mg/l. With an increase in cytokinin up to 2.0 mg/l, a tendency to increase the proliferation coefficient was observed. A positive average correlation was found between such traits as the size of the cutting and the proliferation coefficient. the exception was Camelot, where the correlation coefficient on the medium with 6-BAP 2.0 mg/l was 0.75. The enrichments used at the proliferation stage had a positive effect on the appearance of the micro seedlings. There was a good turgor; high leaf coverage of microcuttings; the leaves were saturated green. The maximum height of micro-seedlings for all varieties was observed in the version with "Siliplant", and the increase was statistically significant ( $p < 0,05$ ) (Table 3).

TABLE III. MICRO-SEEDLINGS HEIGHT OF CLIMBING ROSES, MM

Rose variety	Growing medium option		
	Control	«Siliplant b», 1 ml/l	Iron, 10 mg/l
PalaisRoyal	22,2±4,6	34,4±17,8 <sup>a</sup>	25,7±9,7 <sup>b</sup>
Camelot	18,4±6,6	22,9±9,0 <sup>a</sup>	14,9±6,0 <sup>ab</sup>
Nahema	15,5±5,5	20,0±7,9 <sup>a</sup>	16,6±5,8 <sup>b</sup>

<sup>a</sup> - the differences are statistically significant compared with the control, b - compared with "Siliplant" option.

Siliplant - containing silicon drug. In addition to silicon (7%) and potassium (1%), the composition includes microelements that are readily accessible to plants in a chelated form: iron, copper, zinc, manganese, cobalt, boron. Fertilizer is developed, registered and made by NNPP "NEST M". The drug stimulates the development of the root and aboveground parts, relieves various stresses, and activates photosynthesis. Strengthens the mechanical resistance of cell walls, has a pronounced fungicidal action, sterilizing fungal spores.

While using a double concentration of iron, the height of the microcuttings did not differ from the control one. The exception was the Camelot variety, where the use of this filling agent had a negative impact on the cuttings growth. Among the three varieties of climbing roses, the biggest growth (12.2 mm) was characterized in the microcuttings of the PalaisRoyal variety, when using the "Siliplant" preparation as microelements.

When calculating the proliferation ratio, it turned out that in general, the Palais Royal variety multiplied worse than the rest (2,13 pcs./cutting in control), despite the good growth of microcuttings (Fig. 2). The maximum multiplication factor was noted for Camelot variety on a medium with double iron concentration and amounted to 3.40 pieces / cutting, but the increase was insignificant compared with the control one ( $p = 0,07$ ). At the same time, for the Camelot and Nahema varieties, the use of iron at a concentration medium 10 mg / l resulted in the formation of very small explants, often unsuitable for subsequent transplantation to the lengthening medium. The use of silicon-containing drug "Siliplant" had no significant effect on the reproduction of roses.

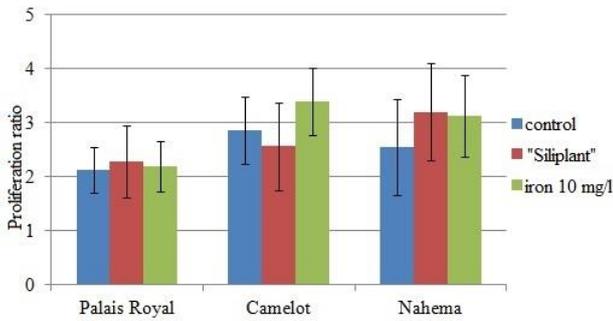


Fig. 2. The proliferation coefficient of climbing rose's varieties

Thus, the use of "Siliplant" drug as microelements had a positive effect on the elongation of the microcuttings of three rose's varieties, while the proliferation coefficient did not significantly differ from the control. The double concentration of iron in the composition of the growing medium led to the breeding of Camelot and Nahema varieties. However, the formed microcuttings were very small, which requires additional research.

The most critical stage in the plants invitro reproduction is the transfer of miniature seedlings from sterile to non-sterile. The factors affecting the viability of microplants during the adaptation period include: substrate type, air humidity, infection load, imbalance between the leaf apparatus and the root system [8].

As a result of research, it was found that the adaptation of rooted invitro climbing rose's microcuttings strongly depended on the genotype. Worst of all, it was held at the PalaisRoyal variety. The success was 76.4%. The Camelot and Nahema varieties on average took root with the same success, 96.6 and 100%, respectively (Table 4).

When studying the effect of the substrate on the success of the adaptation of the micro seedlings, a 100% survival rate of the studied rose varieties on the soil mixture consisting of highmoor and transitional peats ("Universalnaya zemlya sadovaya" ("Universal planting soil")) was noted. After a few weeks, the plants had an attractive appearance with a good turgor of leaves and petioles; the leaves were intensely green. When using the commercial substrate "Torfolin A", the adaptation

TABLE IV. SUCCESSFUL ADAPTATION OF ROSE SEEDLINGS, %.

Variety, Factor A	Options soil mixture, factor B.			Medium
	Laboratory (K)	«Torfolin A»	«Universalnaya zemlya sadovaya»	
PalaisRoyal	35,7	93,5	100,0	76,4
Camelot	93,0	96,8	100,0	96,6
Nahema	100,0	100,0	100,0	100,0
Среднее	76,2	96,8	100,0	
HCP <sub>05</sub>	cut. A	6,2		
	main A	3,6		
	main B	2,4		

The lowest rates were obtained on laboratory soil mixture, on average for three varieties of 76.2%. In PalaisRoyal variety 35.7% of the micro seedlings adapted to environmental conditions, and in Camelot and Nahema varieties - 93.0 and 100.0%, respectively. After the

adaptation period, the micro seedlings were characterized by weak growth, low foliage, and insufficient turgor. In order to avoid death, they were later transplanted to rearing on the "Universalnaya zemlya sadovaya".

When transplanting adapted roses from micropairs in a 0.5 l container, 30 days later, grown shoots and a well-branched root system were noted.

Thus, for the adaptation of rose micro seedlings, a soil-based mixture based on highmoor and transitional peat with 100% success in adapting three varieties of climbing roses turned out to be optimal in physicochemical properties. Among the roses studied, the PalaisRoyal variety was the worst adapted.

IV. CONCLUSIONS

An innovative technology for the production of planting material based on microclonal propagation invitro of three climbing rose's varieties is proposed. On the basis of the conducted studies, it is possible to note high variety specificity at the stages of proliferation and adaptation. The optimal concentration of cytokinin 6-BAP at the stage of proliferation of 1 mg/l was revealed. The introduction of Siliplant into the growing medium contributed to the improvement of the quality of microcuttings, without affecting the proliferation rate. Для получения качественных черенков для этапа ризогенеза на среде для удлинения глюкоза (20 г/л) является лучшим углеводом, чем сахароза, различия статистически достоверны. To obtain high-quality cuttings for the stage of rhizogenesis on a medium for the extension of glucose (20 g / l) is a better carbohydrate than sucrose. The differences are statistically significant. The optimal soil substrate for the adaptation of rose seedlings is a physico-chemical-balanced soil mixture based on highmoor and transitional peat.

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