European Proceedings of
Multidisciplinary Sciences
EpMS

www.europeanproceedings.com
e-ISSN: 2421-826X

DOI: 10.15405/epms.2024.09.83

MTMSD 2022
I International Conference «Modern Trends in Governance and Sustainable Development of Socio-economic Systems: from Regional Development to Global Economic Growth»

THE BIOFERTILIZERS FOR THE ROOT SYSTEM OF APPLE ROOTSTOCKS

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Abstract

The paper presents the results of research on the optimization of the main elements of adaptation and rearing of apple rootstocks in vivo. As a result of the conducted research, the main elements of the technological process of adaptation of apple rootstock plants M-9 and B-9 have been optimized. A consistent complex of nutritional regime is proposed for the effective growth and development of the root system of apple rootstocks, starting from the withdrawal of micro-plants in non-sterile conditions, up to the moment of forcing plants from the conditions of film greenhouses into field ones. The influence of a nutrient Knop's solution and biofertilizers "Lignohumate", "Samorod" and "Orengum" on the development, adaptation and rearing of apple rootstocks obtained in vitro in greenhouses has been studied. It was found that the use of biofertilizers had a significant impact on the formation and development of the root system, activation of growth processes and contributed to strengthening the protective properties of plants, as well as increasing resistance to adverse environmental conditions. The results obtained in the study can be used to work with the culture of cells and tissues of apple plants, in plant biotechnology and nursery, as well as in fruit growing to obtain a homogeneous healthy planting material of woody plants.

Keywords: Apple rootstocks, adaptation to in vivo conditions, in vitro culture, microclonal reproduction

https://doi.org/10.15405/epms.2024.09.83
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eISSN: 2421-826X

1. Introduction

The apple tree is one of the most popular and profitable fruit crops on the world market, its importance is difficult to overestimate. However, from year to year, the number of diseases that reduce the yields of this crop increases and the system of protective measures does not always justify the invested costs. Therefore, many advanced farms in the country and the republic, including, have switched to obtaining planting material by the method of isolated cells and tissues, which guarantees the purity of plants from pathogenic microorganisms, provided that the soil is pretreated from pathogenic microflora. At the same time, in order to maintain the purity of high-yielding agricultural plants, it is desirable to introduce in vitro culture and use healthy planting material with each renewal of such plantings.

Cell and tissue culture is widely used to obtain a large amount of homogeneous healthy basic planting material of valuable crops. It is particularly successful for the propagation of high-yielding fruit trees and grapes in agriculture (Khamurzaev et al., 2022; Sobralieva et al., 2021).

The presence of indole butiric acid in the medium at a concentration of 2 mg/l causes the formation of hypertrophied roots (Van-Unkan et al., 2015). At this stage, it is also advisable to use a nutrient medium depleted in mineral composition. In most cases, this is 50% of the total concentration of the components of the nutrient medium used.

It is known that the apple tree, when introduced into culture in vitro, secretes phenolic compounds that lead to the death of explants. To reduce the concentration of phenols and to avoid the development of tissue necrosis, seedlings are transferred daily to a fresh nutrient medium (Brooks & Driver, 1991; Romadanova et al., 2016).

The stage of adaptation and growth of test tube plants during microclonal reproduction is one of the most critical periods for fruit crops, including apple rootstocks. The rate of rooting, the power of development of the rhizogenic zone, the volume and rate of growth of the vegetative mass largely depends on the genetic potential of the variety, the biology of its development, as well as on the nutritional composition of the substrate (Van-Unkan et al., 2015).

2. Problem Statement

The production of high-quality apple rootstocks is crucial for successful fruit cultivation, and optimizing the adaptation and rearing process of apple rootstock plants is essential for ensuring their successful growth and development. However, there is a lack of comprehensive research on the optimization of the main elements of adaptation and rearing of apple rootstocks, particularly with regards to the use of biofertilizers.

3. Research Questions

The main research questions that the author's collective addresses are:

i. What are the main elements of the technological process of adaptation of apple rootstock plants M-9 and B-9?

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ii. How can the nutritional regime be optimized for the effective growth and development of the root system of apple rootstocks?

iii. What is the influence of Knop's solution and biofertilizers "Lignohumate", "Samorod" and "Orengum" on the development, adaptation and rearing of apple rootstocks obtained in vitro in greenhouses?

iv. How do biofertilizers impact the formation and development of the root system and activation of growth processes in apple rootstocks?

v. Can the results obtained in the study be used to obtain a homogeneous healthy planting material of woody plants and improve fruit cultivation?

4. Purpose of the Study

The main purpose of the research was to study biofertilizers to optimize the nutritional regime of the root system in apple rootstocks on the example of test tube plants B-9, M-9 at the stage of adaptation of micro-plants.

The study aims to propose a consistent nutritional regime for effective growth and development of the root system of apple rootstocks from the withdrawal of micro-plants to forcing plants into field conditions. Ultimately, the results of this research can be used to improve fruit growing by obtaining homogeneous healthy planting material of woody plants and enhancing the protective properties of the plants, as well as expanding the application of these findings in plant biotechnology, cell and tissue culture and nursery production.

5. Research Methods

In the work with cell culture, apple rootstocks M-9 and B-9 were selected. The selection of plant material was carried out during the period of active growth of shoots from visually healthy apple rootstock plants. Sterilization of plant material was carried out with sodium hypochlorite 25%, isolated meristems were planted on a nutrient medium MS supplemented with glycine (0.05 mg/l) and aucin of indole butiric acid– 0.5 mg/l.

The research was carried out according to generally accepted methods when working with cell and tissue culture (Butenko, 1964) in fruit growing, mathematical and statistical processing was carried out by the dispersion method (Dospekhov, 1985) and using the programs Listomer, Biostat and Microsoft Excel. The repetition of the experience is threefold.

6. Findings

The rooting stage took place within 6 weeks, test tube plants with a height of 5-10 cm, a root length of 2 cm to 7 cm and a number of roots of 3-5 pcs. were transferred to in vivo conditions in plastic cups of 800 ml. Thermally disinfected peat, sand and soil (1:1:1) were used as a nutrient substrate. The first treatment of plants was carried out with a Knop's solution, for comparison, in the control version, the

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treatment was carried out with water. The survival rate of plants was taken into account on the 14th day after their transfer to non-sterile conditions of the nutrient substrate (Table 1).

Table 1. Survival rate of micro-plants of apple rootstocks (M-9; B-9)

Apple tree rootstocks	Micro-plants	The number of plants that have taken root, pcs.	
		Control - water	Option 1 – Knop's solution
B-9	Number of plants	30	40
	Number of live plants after 14 days (%)	14	24
	Fraction, %	47	60
M-9	Number of plants	25	64
	Number of live plants after 14 days (%)	13	51
	Fraction, %	52	79

From the data given in Table 1, it can be seen that the survival rate of micro-plants of apple rootstocks is higher when using the Knop's solution. Thus, the best result was noted on the variant using the Knop's solution, where the survival rate of the B-9 rootstock was 60% and the M-9 rootstock was 79%.

In addition, at the stage of adaptation to ex vitro conditions, the effect of potassium Lignohumate biofertilizer on the survival rate of apple rootstocks was studied. The choice of the preparation is based on its ability to increase the resistance of plants to adverse environmental factors – the preparation has the effect of a fertilizer and a growth regulator at the same time. The treatment of the root system was carried out beforehand before planting micro-plants and watering an aqueous solution of the substrate after. In order to establish the optimal effect, various concentrations of the preparation were studied: 0.5 g/l; 1.0 g/l; 2.0 g/l. Water was used as a control variant.

During the observations, it was found that potassium Lignohumate at specified concentrations had a positive effect on plants, including an increase in plant height and leaf area. The best results in increasing the height of plants were noted on the 30th day of adaptation, at a preparation concentration of 1.0 g/l. (Table 2).

Table 2. The use of potassium Lignohumate in the adaptation of micro-plants of apple rootstock B-9 to non-sterile conditions *in vivo*

Accounting time, days after trial establishment	Concentration of Lignohumate, g/l	Stem height, cm	Number of leaves, pcs.	Number of roots, pcs.	Root length, cm	Growth rate per day, cm
10	Control	7.0	5.8	4.0	7.1	1.40
	0.5	9.8	8.7	5.1	7.3	1.71
	1.0	12.8	10.9	5.0	8.7	2.15
	2.0	14.2	12.0	5.2	7.4	2.16
20	Control	10.2	7.9	4.0	8.6	0.94
	0.5	18.2	16.0	4.9	8.9	1.35
	1.0	23.0*	18.2	5.1	11.5	1.70
	2.0	25.6	18.8	5.3	8.4	1.70

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30	Control	15.6	10.4	4.5	8.6	0.80
	0.5	32.6	18.5	4.9	10.2	1.40
	1.0	35.2	18.6	5.0	14.5	1.66
	2.0	38.8	20.2	5.1	10.4	1.64

Micro-plants of apple rootstocks, when adapting to in vivo conditions with low humidity, had a slight wilting, then a slight drying of the edges of the leaf blades of the lower tier of leaves was observed. Most likely, this is due to the fact that it is more difficult for old leaves to rebuild the transpiration system than for younger ones on the upper tiers of apple rootstock plants. Despite such changes in the development of the leaf apparatus of plants, which is not directly related to the use of the preparation itself, in general, the use of the preparation had a positive effect on the development and adaptation of test tube plants of apple rootstocks. Thus, the most effective for the B-9 rootstock were variants with a concentration of potassium Lignohumate of 1.0 g/l and 2.0 g/l. It should be noted that plants with concentrations of 1.0 g/l and 2.0 g/l looked much stronger, had higher biometric characteristics, and plants with shrinking edges of leaf blades were less common among them.

Table 3. The use of potassium Lignohumate in the adaptation of micro-plants of apple rootstock M-9 to non-sterile conditions *in vivo*

Accounting time after trial establishment, days	**Concentration of Lignohumate, g/l**	**Stem height, cm**	**Number of leaves, pcs.**	**Number of roots, pcs.**	**Root length, cm**	**Growth rate per day, cm**
10	Control	7.8	4.1	4.5	6.0	1.38
	0.5	9.9	8.0	5.1	6.9	1.68
	1.0	13.1	9.9	6.0	7.7	2.08
	2.0	14.5	11.5	6.2	8.4	2.29
20	Control	11.0	8.9	4.9	8.6	0.98
	0.5	17.5	13.0	5.5	8.9	1.32
	1.0	20.0*	18.2	5.5	11.5	1.50
	2.0	19.6	18.8	5.3	10.4	1.50
30	Control	15.4	10.1	5.5	10.3	0.80
	0.5	27.6	15.5	6.0	12.4	1.30
	1.0	33.6	17.1	6.1	13.9	1.60
	2.0	34.4	19.0	5.3	13.3	1.59

The data given in Table 3 indicate similar results of the development of apple rootstocks M-9 with rootstock plants B-9, while, as in the first case, the use of concentrations of 1.0 g /l and 2.0 g/l shows the best biometric development results.

Thus, when using a concentration of potassium Lignohumate 0.5 mg/l, the deviation from the control version of the experiment was not significant. On average, the height of the stem was 9.9 cm, the number of leaves was 8.0 pcs., the number of roots was 5.1 pcs., the length of the roots was 6.9 cm and the growth rate per day was 1.68 cm. At the same concentration, after 20 days of plant adaptation, significant changes were noticeable, the height of the stem increased by 7.6 cm, the number of leaves by 5 pcs., the number of roots by 0.4 pcs., the length of the roots by 2 cm, but the growth rate per day

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decreased by 0.36 cm. And accounting after 30 days of plant adaptation showed 27.6 cm, 15.5 pcs., 6.0 pcs., 12.4 cm, 1.30 cm – according to biometric indicators.

An increase in the concentration of potassium Lignohumate in subsequent versions of the experiment contributed to the improvement of the analyzed indicators, so the best results on the 30th day of accounting for adapted plants at concentrations of 1.0 mg/l and 2 mg/l were as follows: the height of the stem was 34.4 cm, the number of leaves was 19.0 pcs., the number of roots was 6.1 pcs., the length of the roots was 13.9 cm and the growth rate per day was 1.6 cm.

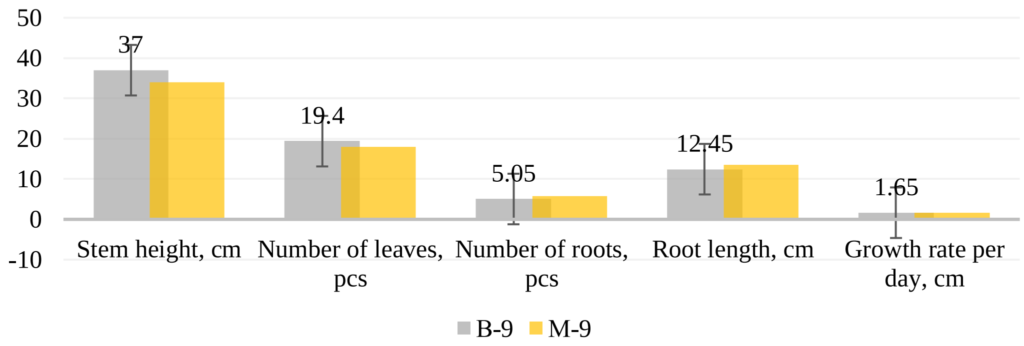

Figure 1. The effect of potassium Lignohumate on the parameters of adaptation of test tube plants of apple rootstocks B-9 and M-9 to non-sterile conditions *in vivo*

In general, the influence of potassium Lignohumate on the development of the M-9 rootstock was more pronounced in the formation of the root system during plant adaptation, visually the M-9 roots looked stronger and more developed. Note that the stem part of the B-9 was developed more powerfully compared to the M-9. Such minor differences in the development of test tube plants of apple rootstocks are rather related to the genotypic features of the rootstocks themselves, rather than with the use of one or another concentration of potassium Lignohumate (Figure 1).

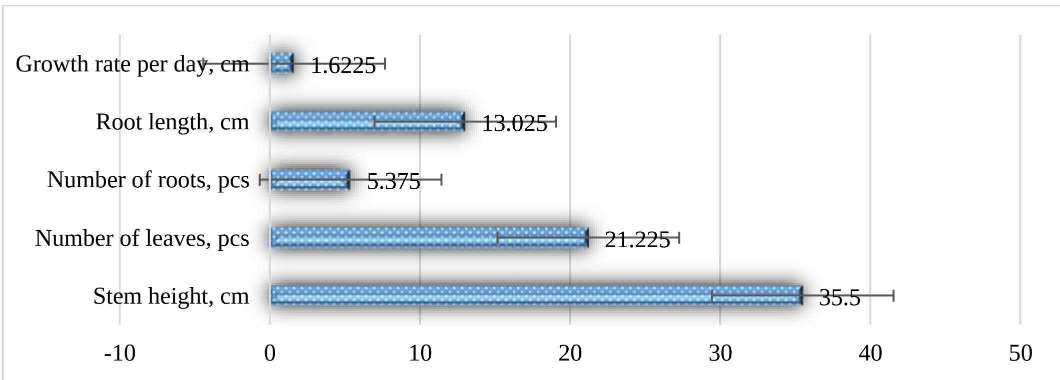

Figure 2. Adaptation and development of apple rootstock plants using the most effective concentrations of potassium Lignohumate

Thus, the studied apple rootstocks B-9 and M-9 had high indicators of biometric development when using the most effective concentrations of potassium Lignohumate on the 30th day of accounting - Figure 2.

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After adapting to non-sterile conditions, for further growing of the apple rootstock plants, they were moved to a film greenhouse in the third decade of May. This period is most effective for good adaptation and development of plants obtained in vitro culture. The planting of plants at a later date was accompanied by their slow development and partial dyewood, due to an increase in air temperature and insufficient humidity. Seedlings were transplanted into vessels (20x10x10 cm) with a soil substrate for rooting (a mixture of sand and soil in a ratio of 1:2). In order to harden and develop resistance to adverse environmental conditions in plants adapted to non-sterile conditions, experiments were conducted to study the effect of biofertilizers Samorod and Orengum on the growth and development of apple rootstocks B-9 and M-9. Concentrations of biofertilizers were selected in accordance with scientifically based data for growing apple seedlings in protected soil (Avdeeva et al., 2018; Panova et al., 2018). Watering with fertilizer solutions was carried out 1 time in 10 days; water was used as control. The experiment was repeated three times, using 30 plants in each variant.

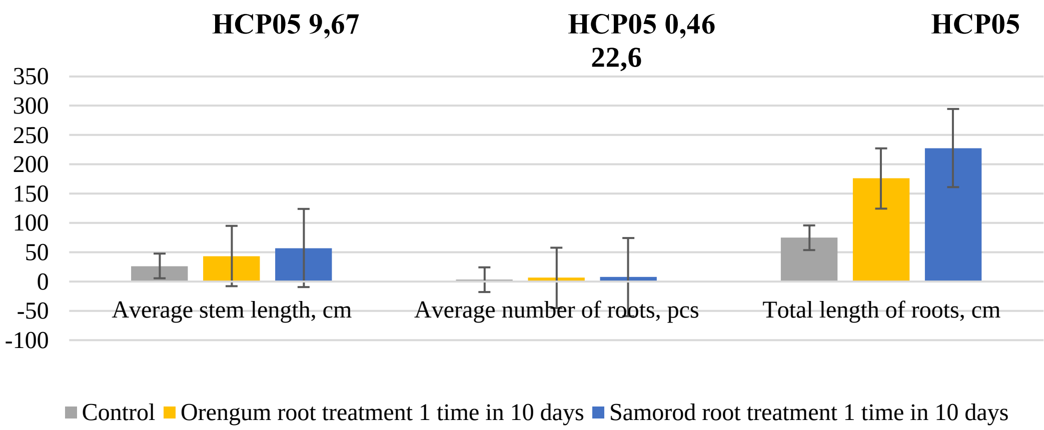

Figure 3. The effect of biofertilizers "Samorod" and "Orengum" on the growth and development of apple rootstock plants obtained in vitro culture after transplanting seedlings for adaptation to protected soil

Thus, the data in Figure 3 indicate that the variant with the use of the "Samorod" biofertilizer most significantly affected the development of apple rootstocks when growing in a film greenhouse. The results of the experiment show that the average length of growth of apple rootstock seedlings under the influence of biofertilization treatment increased from 26.7 cm to 57.3 cm, the average number of roots – from 3.2 pcs. to 7.6 pcs. and the total length of the root zone increased compared to the control version of the experiment from 74.8 cm to 227.9 cm.

The actual deviation from the control when measuring the average length of plant growth of apple rootstocks M-9 and B-9 when treated with "Orengum" biofertilizer was 16.9 cm, while when treated with "Samorod" biofertilizer, the deviation of this indicator was 30.6 cm. When comparing the average number of roots with the indications of the control variant, the deviation value on the "Orengum" variant was 3.1 pcs., and on the "Samorod" variant - 4.4 pcs. In terms of the total length of the roots, the deviation from the control of the experiment was 101.1 cm when using "Orengum" and 153 cm when using "Samorod". Thus, the use of biofertilizers "Orengum" and "Samorod" had a positive effect on the development of apple rootstocks M-9 and B-9 when growing seedlings in greenhouse conditions, activating endogenous growth processes and rooting plants.

7. Conclusion

The results obtained make it possible to optimize the main elements of the technological process of adaptation of M-9 and B-9 plants obtained from meristematic explants under in vitro conditions:

For the adaptation of apple rootstocks M-9 and B-9 obtained in vitro culture to non-sterile conditions in vivo in the first two weeks of plant adaptation, it is advisable to use a Knop's solution as irrigation, while the survival rate of the B-9 rootstock is 60% and the M-9 rootstock is 79%. The use of potassium Lignohumate in the next 30 days of adaptation (in concentrations of 1 g/l or 2 g/l) makes it possible to obtain strong seedlings of apple rootstocks with high biometric indicators for further growth. When transferring plants after adaptation to the conditions of a film greenhouse for growing, the use of biofertilizer "Samorod" is effective, which has a positive effect on plant development by activating endogenous growth processes to accelerate the development of the root system of plants. It is also established that the most optimal period for transferring and planting apple rootstocks adapted to non-sterile conditions is the last decade of March, we note that this is the most optimal period with the least dyewood of plants.

As a result of our research, we have improved biotechnological methods of rooting, adaptation and rearing of apple rootstocks M-9 and B-9 in the technology of clonal micro-propagation in culture in vitro, providing mass production of planting material taking into account the following features:

i. the reproduction rate – 3-5;
ii. *in vitro* cultivation cycle – 30-40 days;
iii. ex vitro cultivation cycle (vegetative vessels) – 4-7 weeks;
iv. the survival rate of adapted plants — 70-90%.

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