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BIOTECHNOLOGICAL METHODS OF POTATO (SOLANUM TUBEROSUM L.) REPRODUCTION IN *IN VITRO* CULTURE USING ELEMENTS OF CHEMOTHERAPY

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ABSTRACT

The elements of chemotherapy for the recovery of potato plants *in vitro* were selected. The influence of various modifications of the nutrient medium on changes in morphological parameters was investigated. The process of reproduction of potato plants of different varieties was studied. It was experimentally proved that *in vitro* potato recovery with elements of chemotherapy ensures the reproduction of the source material on a virus-free basis by inhibiting the activity of viruses and bacteria with virus inhibitory and antibacterial drugs. The absence of viruses in the obtained material is confirmed by the results of enzyme-linked immunosorbent assay (ELISA) diagnostics. Studies have shown that the use of Noviryn is the most effective element of chemotherapy for Serpanok and Skarbnytsia varieties. Its action contributed to an increase in plant height and the number of leaves per shoot, and increased the multiplication factor to 4.5 and 3.9, respectively. The use of Noviryn in combination with Dekasan is the best option for improving Mezhyrichka 11 variety and leads to the improvement of certain morphological parameters (+4.5 cm of stem length, +3.5 of leaves) and the multiplication coefficient (+1.0). The use of Noviryn in combination with Salicylic Acid had a positive effect on changes in the morphological parameters of all potato varieties under study. It should be noted that during the growing season in the field, the first field generation of *in vitro* plants was resistant to secondary infection with viral diseases.

Keywords: potato, nutrient medium, chemotherapy, meristematic tissue culture, regeneration, morphogenesis *in vitro*

INTRODUCTION

For Ukraine, potato is one of the crops that ensure its food security. Of course, crops such as sunflower, wheat and corn exceed most fodder and food crops in terms of cultivation area **(Vasylkovska K. *et al.*, 2021; Mostipan M. *et al.*, 2021; Vasylkovska K. *et al.*, 2022)**. However, potato occupies an influential place in the diet of an average Ukrainian. The main regions of industrial potato production are the Central and Northern regions, but tubers are grown in almost all regions of the country, regardless of climate conditions and farm size.

Potato (*Solanum tuberosum L.*) is an extremely important food crop. It is grown in 150 countries around the world, for many of which it is the staple food. On an industrial scale, it is propagated vegetatively. However, this method of propagation leads to the accumulation of fungal, bacterial and viral pathogens in the planting material. This not only reduces crop productivity, but also threatens the preservation of genotypes for commercial or breeding purposes. Due to the impossibility of using botanical seeds for propagation, germplasm exchange of this species does not occur naturally, which leads to the degeneration of potato varieties. In the countries where potatoes are grown, the demand for high-quality planting tubers is constantly growing, and their production is a priority for ensuring industrial cultivation of this crop **(Basera, M. *et al.*, 2018; Bamberg J. B. *et al.*, 2016).**

Currently, about 50 phytopathogenic viruses have been identified on potatoes. The most harmful among them are potato leaf roll virus, PLRV; Y-virus, Potato virus Y; X-virus, Potato virus X; S-virus, Potato virus S; M-virus, Potato virus M; A-virus, Potato virus A; Potato aucuba mosaic virus; Potato mop-top virus; Tomato black ring virus; Potato yellow dwarf virus **(Mulema J. *et al.*, 2021; Oliinyk T. *et al.*, 2012; Bradshaw J. *et al.*, 2007; Kumar R., *et al.*, 2019).**

The first four of these are considered most common and harmful. Nutrient medium of various modifications for virus-free potatoes opens the way for maintaining high-quality source material for breeding. A combination of field methods for selecting the best clones and laboratory methods for restoring planting material using apical meristem culture, thermotherapy, and suppression of virus replication with chemicals most effectively addresses the issue of renewal and improvement of potato varieties **(Aksoy E. *et al.*, 2021).**

Modern biotechnology offers three main *in vitro* therapies. They are meristem culture, cryotherapy, and chemotherapy combined with thermotherapy that reduce the activity of virus pathogens or even destroy them altogether **(Zhang Z. *et al.*, 2019).**

The apical meristem culture method is the main therapeutic method for treating plants and freeing them from virus infection. This method is widely used to improve potatoes, strawberries, cloves and some fruit and berry crops. But the conditions for cultivating meristems have been developed in the most detail for potatoes. Considering that viruses are constantly changing and becoming more harmful under the influence of the environment, there is a need to find more effective measures to improve potato plants. The combination of apical meristem culture and chemotherapy to produce virus-free material is more radical and is used even in the case of severe virus infection. The use of chemotherapy makes it possible to use a larger meristem (200 µm) and achieve a healthier result. The use of virus-inhibiting compounds stimulates the survival of meristems by 10-35%, and the yield of asymptomatic meristem plants is 80-100%, depending on the characteristics of the variety. The most effective recovery of the source material is when, in addition to the combination of apical meristem culture and chemotherapy, thermotherapy is used **(Sarkar D. & Naik P. S., 1998).**

Therefore, biotechnological methods based on tissue culture are very important and relevant. Plant tissue culture enables the use of alternative *in vitro* propagation methods that provide rapid reproduction of healthy, high-quality planting material. According to some authors, the use of these technologies in potato production chain directly benefits both producers by providing high productivity of pathogen-free potato tubers and consumers of these products **(Morais, T.P. *et al.*, 2018; Qadri R.W.K. *et al.*, 2021).**

However, the unresolved problematic issues of creating virus-free potato planting material hinder the effective use of biotechnological methods. These issues include, for example, the risks of mass infection of plants *in vivo* due to undetected latent infections in meristem material, and the preservation of variety identity and yield properties at the level of its genetic potential during the recovery process. Thus, along with monitoring the virological status (enzyme-linked immunosorbent assay or polymerase chain reaction), it is necessary to identify recovered lines and varieties both at the DNA level and by morphological characteristics.

The process of *in vitro* regeneration depends primarily on the variety characteristics. It has been proven that plant height can range from 45.5 mm to 123.7 mm. At the same time, variety characteristics under *in vitro* conditions determine the growth and development of leaves, the number and length of internodes, stem thickness, root formation and growth, the beginning and intensity of stolon and tuber formation, etc. **(Oliynyk T. M. *et al.*, 2013; Shahzad A. *et al.*, 2017; Sharma S. K. *et al.*, 2007; Ali S. *et al.*, 2018; Bamberg J. B. *et al.*, 2016;**

Dodds J. H. *et al.*, 1992; Nagib A. *et al.*, 2003; Naik P. S. & Buckseth T. 2018; Venkatasalam E. P. *et al.*, 2015; MatskevychV. V., 2020).
Modern biotechnology methods use antibiotics, enzymes, metabolic products of yeast, fungi and bacteria, the juice of certain plants (Panax ginseng, Aloe arborescens, Agáve), amino acids, nucleic acids, dyes, phytohormones, etc. as virus inhibitors. The most commonly used antiviral chemotherapeutic agents are nucleoside drugs or their analogues. A standard substance widely used for potato varieties recovery is ribaviryn or virazol (1-β-D-ribofuranosyl-1, 2, 4-triazole-3-carboxamide), a nucleoside analogue with antiviral activity against many plant viruses, including potato viruses **(Streeter D. G. et al, 1973; Oliynyk T. M. *et al.*, 2013).** New directions of chemotherapy of viral infections are promising, which cause damage to viral genes or inhibit the activity of viruses. Compounds with such effect include abnormal nucleosides, pyrimidine and purine analogues, adamantanamine hydrochloride derivatives, etc.
It should also be noted that there is still no universal method for assessing the toxicity and antiviral activity of substances used for plant recovery *in vitro* **(Harina A. V. *et al.*, 2003).** In addition, the concentrations and doses of most chemicals are not determined, and the terms of influence of antiviral and antimicrobial drugs not only on suppression of viral and bacterial infections, but, most importantly, on the formation of resistance of plant organisms to pathogens remain unexplored. Special attention should also be paid to the study of potato variety characteristics, which are an important factor in determining the elements of chemotherapy in combination with meristem culture.

MATERIAL AND METHODS

The main objective of the research was to determine the effect of Noviryn, Dekasan and Salicylic Acid (Tab. 1) on the morphogenesis of potato plants *in vitro* by introducing them into the culture medium.
The study of the effect of the above-mentioned elements of chemotherapy *in invitro* on the growth, development and release of different potato varieties (Mezhyrichka 11, Serpanok, Shchedryk, Skarbnytsia) (Tab. 2) from viral infections was carried out in the laboratory of bio-adaptive technologies of the Institute of Agriculture of the National Academy of Agrarian Sciences of Ukraine. The material of apical meristems of the Institute of Potato Growing of the National Academy of Agrarian Sciences of Ukraine was used in the *in vitro* culture.
The studied elements of chemotherapy were used in the following doses: Novyrin – 20 mg/100 ml solution and Dekasan – 2.5 ml/100 ml solution are provided as their physical weight. In terms of the amount of active substance, this corresponds to: 20 mg of inosine pranobex (Novyrin preparation) per 100 ml of solution; 0.5 mg of decamethoxin (Dekasan preparation) per 100 ml of solution. The specified dosage of salicylic acid – 1.14 mg/100 ml solution, represents the amount of active substance. They were introduced into Murashige-Skoog nutrient medium (control) in the modification of the Institute of Potato Growing of the National Academy of Agrarian Sciences of Ukraine.

Table 1 Characteristics of the studied elements of chemotherapy

Name	Characteristics
Noviryn	Antiviral drug with antiviral effect caused by binding to the ribosomes of virus-infected cells and leading to inhibition of replication of RNA- and DNA-genomic viruses
Dekasan	The drug has antimicrobial and antifungal action, has a fungicidal effect on yeast, yeast-like fungi, some types of moulds *(Aspergillus, Penicillium)*
Salicylic Acid	Antiseptic agent, inhibits the growth of microorganisms and fungi. Promotes temperature increase in certain organs. Its role in the development of a nonspecific response to stressors and the accumulation of reactive oxygen species in cells is being actively studied

Chemotherapy *in vitro* culture was started from the second passage and continued for the next two passages. During both passages, the following morphological parameters of *in vitro* plants were determined: height from the root collar, number of nodes, internodes, leaves; number of cuttings obtained.
Cultivation was carried out in cultivation rooms at the temperature of 24 ± 2°C, illumination of 3000-4000 lux, and relative humidity of 65-70%. The rooted plants were transplanted into individual containers and kept indoors for 10-14 days, after which they were placed in the field.
Diagnostics for the content of virus antigens by enzyme-linked immunosorbent assay (ELISA) was performed in the laboratory of the Institute of Potato Growing of the National Academy of Agrarian Sciences of Ukraine twice: before the release of microplants from tubes and after flowering.

Table 2 Characteristics of the potato varieties under study

Name	Characteristics
Mezhyrichka 11	The patent applicant and owner of the variety is the Institute of Potato Growing of the National Academy of Sciences of Ukraine. The variety is of medium early maturity group of table variety. It is included in the State Register of Plant Varieties Suitable for Distribution in Ukraine 2014
Serpanok	The patent applicant and owner of the variety is the Institute of Potato Growing of the National Academy of Sciences of Ukraine. The variety is of early maturity group, of table variety. It was included in the State Register of Plant Varieties Suitable for Distribution in Ukraine in 2001
Shchedryk	The patent applicant and owner of the variety is the Institute of Potato Growing of the National Academy of Sciences of Ukraine. The variety is of early maturity group, of table variety. It is included in the State Register of Plant Varieties Suitable for Distribution in Ukraine 2011
Skarbnytsia	The patent applicant and owner of the variety is the Institute of Potato Growing of the National Academy of Sciences of Ukraine. The variety is of early maturity group, of table variety. It was included in the State Register of Plant Varieties Suitable for Distribution in Ukraine in 2008

In order to analyse the data received, statistic models and a package of Excel Microsoft Office applications were used **(Vasylkovskyi O. *et al.*, 2016)**.

RESULTS AND DISCUSSION

Cuttings taken from the middle part of the stems were introduced into the *in vitro* culture of the potato varieties under study. The bud meristems formed in this part of the stems are similar in shape to those from the apical bud, and at the same time they have almost the same regenerative properties *in vitro*. Before the start of chemotherapy, the cuttings obtained from the mother plant were cultured for two passages. The peculiarities of morphogenesis of different potato varieties *in vitro* culture were determined. As a result, it was found that the studied varieties differed in the main parameters (Fig. 1).

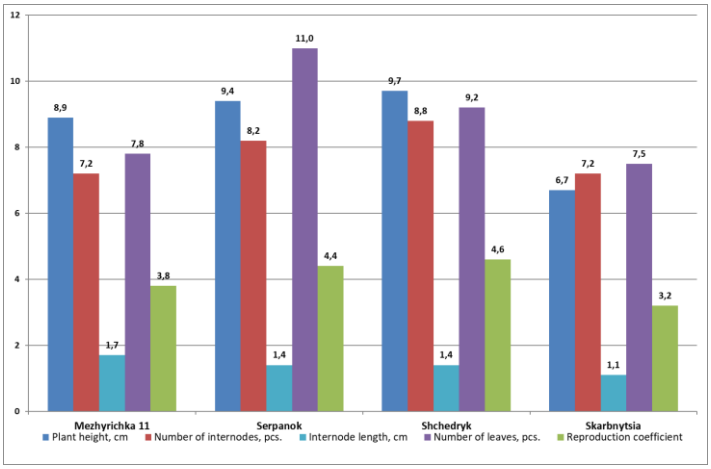


Figure 1 Characteristics of plants of different potato varieties *in vitro* culture before the use of chemotherapy

The most intensive organogenesis *in vitro* occurred in Serpanok plants. They formed an average of 11.0 leaves per stem. The height of plants of this variety did not exceed 9.4 cm; the stem consisted of 8.2 internodes. Two leaves were formed in the nodes of the middle part of the stem. Plants of Shchedryk variety were the tallest (9.7 cm) and had the largest number of internodes (8.8 pcs.) with an average length of 1.4 cm. The peculiarity of this variety was the formation of complex leaves with the largest photosynthetic area during *in vitro* organogenesis. The plants of Mezhyrichka 11 variety formed 7.8 leaves per shoot *in vitro*, and therefore this variety is characterised as well leafed *in vivo*. The regeneration of Skarbnytsia plants was slow, the average plant height for two passages was 6.7 cm, the stem consisted of 7.2 internodes, and the nodes had 7.5 small leaves. A larger number of cuttings were obtained from mother plants of Shchedryk variety, with a multiplication factor of 4.6. Plants of Serpanok and Mezhyrichka 11 were multiplied 1:4.4 and 1:3.8, respectively. The lowest number of cuttings was obtained from Skarbnytsi plants - 3.2.

Thus, the best morphological parameters *in vitro* were characterised by microplants of Serpanok and Shchedryk varieties. The peculiarity of Skarbnytsia variety is its slow regeneration and morphogenesis when cultivated in Murashige-Skoog medium of the modification of the Institute of Potato Growing of the National Academy of Agrarian Sciences of Ukraine.

The introduction of virus inhibitory and antibacterial compounds into the nutrient medium affected the process of reproduction and induction of morphogenesis of potato varieties *in vitro* culture. Plants regenerated on the media of different modifications differed in height, number and size of nodes, internodes and leaves. Thus, the use of Noviryn in the first passage in microplants of Serpanok and Skarbnytsia varieties had a positive effect on their height, number of leaves and multiplication rate (Fig. 2).

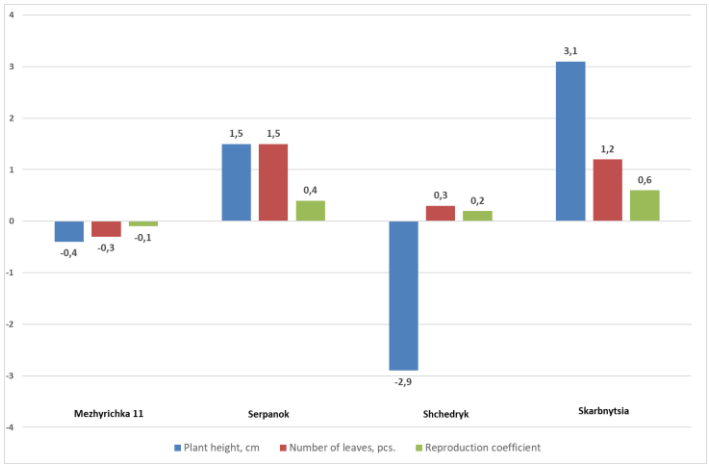
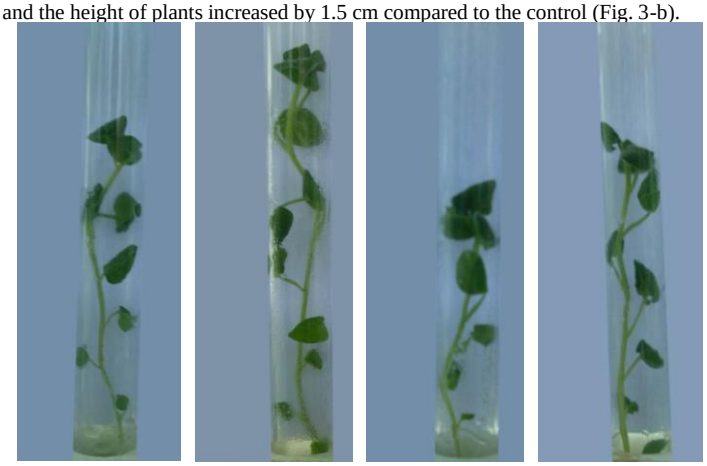


Figure 2 The effect of Noviryn on the intensity of organogenesis *in vitro* culture of different potato varieties, first passage (+/- to the control)

More active morphogenesis was observed in plants of Serpanok variety. Nodes were set more frequently, the number of leaves increased by 1.5 pieces on average, and the height of plants increased by 1.5 cm compared to the control (Fig. 3-b).



a **b** **c** **d**

Figure 3 Morphological features of Serpanok variety on different modified media: a) Control; b) Control + Noviryn; c) Control + Noviryn + Dekasan; d) Control + Noviryn + Salicylic Acid

The plants of Skarbnytsia variety were distinguished by intensive shoot growth. Their height exceeded the plants of the control variant by 3.1 cm (Fig. 4-b)

A specific feature of Shchedryk variety was slow shoot growth and intensive organ formation (Fig. 5-b). At the height of only 6.3 cm (-2.9 cm to the control), the plants had an average of 9.5 leaves, and in some plants, even the formation of lateral shoots was observed. This is the only variety that formed compound leaves *in vitro* culture. In addition, their linear dimensions and area were the largest among the studied varieties, regardless of the modification of the environment.

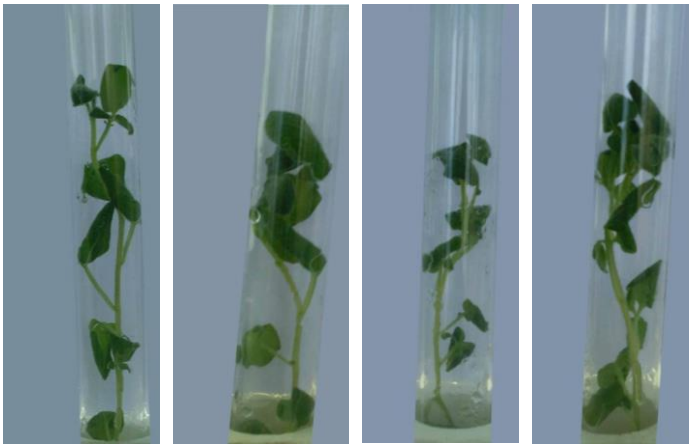
The use of Noviryn had a negative effect on the regeneration of plants of Mezhyrichka 11. A decrease in plant height to an average of 8.5 cm (-0.4 cm), the number of leaves to 7.5 (-0.3), and a decrease in the multiplication coefficient to 3.7 was observed compared to the environment in which this drug was not used (Fig. 6-b). In the control, plants of Serpanok and Shchedryk varieties had the highest multiplication coefficient 4.8, but under the influence of Noviryn, the growth of this indicator was lower than in other varieties and was 0.4 and 0.2, respectively.

Thus, the use of Noviryn in the culture medium contributed to an increase in the height and number of leaves *in vitro* plants and an increase in the multiplication rate in Serpanok and Skarbnytsia varieties.

The combined use of Noviryn and Dekasan in the culture medium had a positive effect on the process of plant regeneration *in vitro* only in Mezhyrichka 11. In other varieties studied, there was a decrease in the height, number of leaves and plant reproduction rate or remained at the same level as in the control variant (Fig. 7).

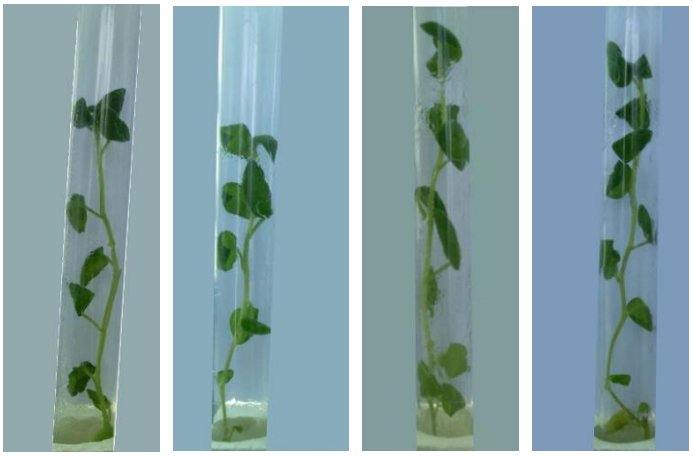
a **b** **c** **d**

Figure 4 Morphological features of Skarbnytsia variety on different modified media:
a) Control; b) Control + Noviryn; c) Control + Noviryn + Dekasan; d) Control + Noviryn + Salicylic Acid



a **b** **c** **d**

Figure 5 Morphological features of Shchedryk variety on different modified media:
a) Control; b) Control + Noviryn; c) Control + Noviryn + Dekasan; d) Control + Noviryn + Salicylic Acid



a **b** **c** **d**

Figure 6 Morphological features of Mezhyrichka 11 on different modified media:
a) Control; b) Control + Noviryn; c) Control + Noviryn + Dekasan; d) Control + Noviryn + Salicylic Acid

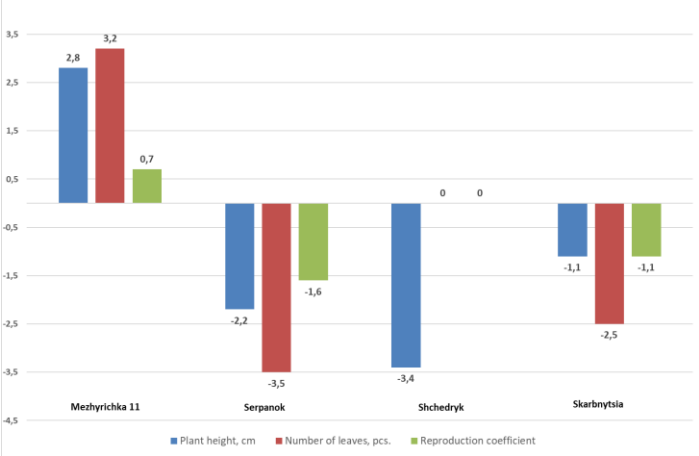


Figure 7 Influence of Noviryn and Dekasan on the intensity of organogenesis in vitro of different potato varieties, first pass, (+/- to the control)

On average, the height of plants of Mezhyrichka 11 was 11.7 cm, which is 2.8 cm more than in the control variant. At the same time, they had elongated internodes with uniform distribution of leaves. Their number averaged 11.0 pieces per plant, which is 3.2 pieces more than in the control (Fig. 6-c).

Shchedryk variety showed a special reaction to the combined addition of Noviryn and Dekasan to the nutrient medium. The height of the plants did not exceed 6.3 cm, while in the control medium the plants grew up to 9.7 cm. Plants were distinguished by shortened internodes, and the number of leaves of much smaller size averaged 9.2 pcs/plant. The multiplication coefficient of plants was at the level of the control variant – 4.6 (Fig. 5-c).

Plants of Serpanok and Skarbnytsia varieties were particularly depressed. The height of their shoots did not exceed 7.5 cm and 5.6 cm, respectively, and they were curved with a small number of small leaves (Fig. 3-c, 4-c). Thus, the simultaneous addition of Noviryn and Dekasan to the nutrient medium negatively affected the growth and development of potato plants of Serpanok, Shchedryk and Skarbnytsia varieties during the first passage.

The addition of Noviryn and Salicylic Acid to the culture medium contributed to positive modifications of morphological traits *in vitro* plants of Skarbnytsia, Mezhyrichka 11, and Shchedryk varieties (Fig. 8).

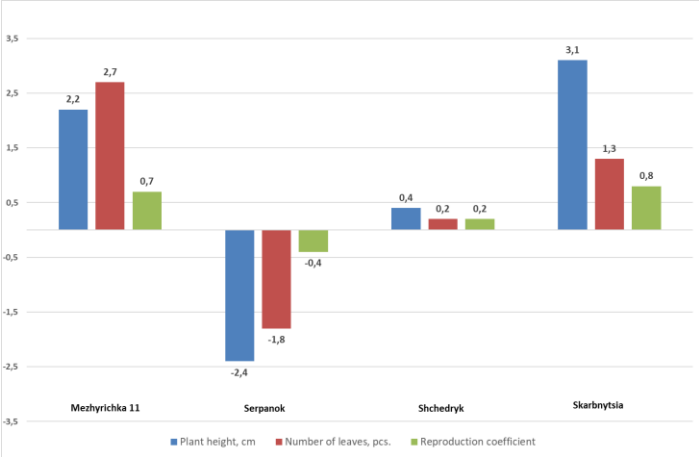


Figure 8 Influence of Noviryn and Salicylic Acid on the intensity of organogenesis *in vitro* of different potato varieties, first passage (+/- to the control)

In these medium, high rates of regeneration of Skarbnytsia plants were recorded at the beginning of cultivation, but after the formation of the fourth leaf, the growth rate of plants slowed down sharply. Therefore, the plants had elongated lower and shortened upper internodes (Fig. 4-d). The growth rate of plants of Mezhyrichka 11 was the same during the first passage. They formed a shoot 11.1 cm high (+ 2.2 cm) with a uniform distribution of leaves (10.5 pcs., + 2.7 pcs./plant to the control variant) (Fig. 6-d). On this medium, minor positive changes in the studied traits were observed in Shchedryk variety. The increase in plant height compared to the control variant was 0.4 cm, and the number of leaves was 0.2 pcs./plant. However, the stems of plants were curved with different internode lengths. At the same time, the activity of organogenesis of plants of this variety was observed after the formation of the fifth leaf (Fig. 4-d).

The potato variety Serpanok, which responded most positively to Noviryn, showed the worst response to the combined effect of Noviryn and Salicylic Acid among the varieties studied. Observations showed an extremely depressed state of plants. Their height was 2.4 cm less than in the control variant and the number of leaves was only 1.8 per plant (Fig. 3-d).

According to the results of the first passage with the use of chemotherapy elements, it was found that the multiplication coefficient of Mezhyrichka 11 and Skarbnytsia varieties increased by 0.7 (4.5) and 0.8 (4.0), respectively. The potato variety Skarbnytsia has a low reproductive capacity *in vitro* compared to other varieties, and the modification of the medium with the addition of Noviryn + Salicylic Acid contributed to the improvement of morphological characteristics and increased the multiplication coefficient of this variety.

In accordance with the research programme, for the second passage, we used culture media with the same drugs as for the first passage. However, it was found that some potato varieties showed a different reaction to the content of the chemotherapy elements we studied in the culture medium (Fig. 9). For example, in Shchedryk variety the intensity of plant regeneration in the medium with the addition of Noviryn was significantly higher than in the control variant, and the plants were 2.2 cm larger. It is worth mentioning that in the first passage on this medium, the opposite reaction was observed and the plants were 2.9 cm shorter than the control variant. At the same time, the number of leaves and the reproduction rate remained unchanged.

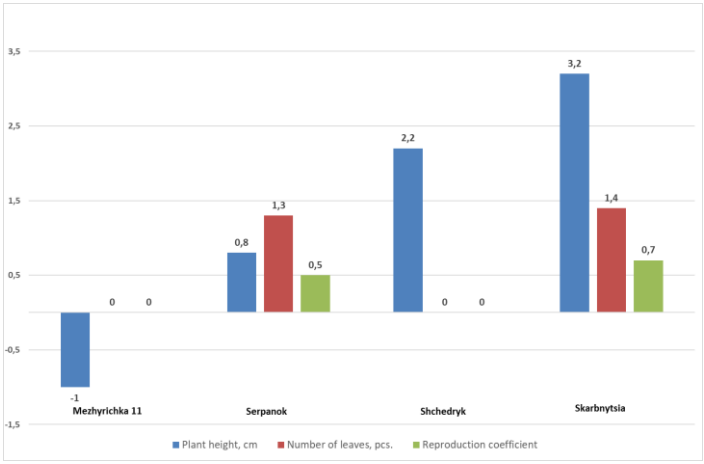


Figure 9 The effect of Noviryn on the intensity of organogenesis *in invitro* culture of different potato varieties, second passage (+/- to the control)

The plants of Skarbnytsia variety on Noviryn medium were distinguished by high growth rates (+3.2 cm) and uniform nodulation and development of leaves; their number increased by 1.4 pcs/plant. In Serpanok variety on this medium during the second passage, the regeneration rates were higher than in the control variant, but slightly lower than in the first passage. Mezhyrichka 11 variety during the second passage, as well as during the first passage, reacted negatively to the content of Noviryn medium.

The reaction of the studied varieties to the combined addition of Noviryn and Dekasan to the culture medium during the second passage of plants *in vitro* was absolutely identical to the reaction observed during the first passage. The varieties Serpanok and Shchedryk reacted negatively to these substances, as evidenced by the height and number of leaves in plants compared to the result obtained in the control variant (Fig. 10). The variety Mezhyrichka 11 had high growth and development rates. The height of plants reached 12.9 cm, which is 4.0 cm more than in the control variant, and the number of leaves exceeded the control by 3.5 pcs.

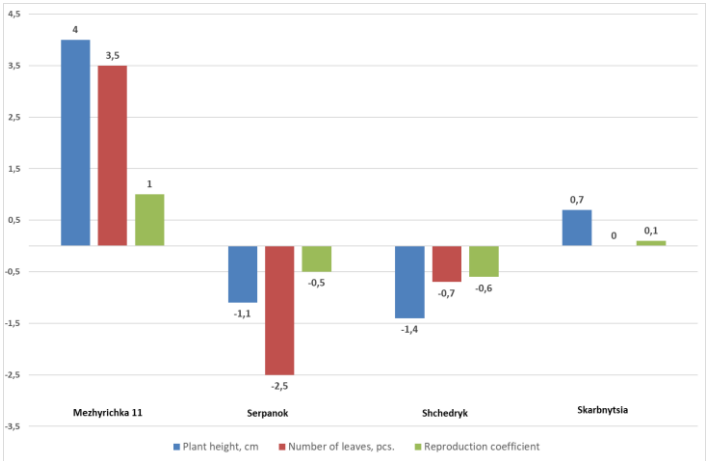


Figure 10 The effect of Noviryn and Dekasan on the intensity of organogenesis *in vitro* culture of different potato varieties, second passage, (+/- to the control)

In Skarbnytsia variety, during the second passage, the opposite reaction of plants to the content of Noviryn and Dekasan in the nutrient medium was found compared

to the first. While during the first passage, plant inhibition was observed, during the second passage, a slight positive reaction was observed. The height of the plants increased by 0.7 cm compared to the control variant and reached 7.4 cm against 6.7 cm. The number of leaves per plant and the multiplication coefficient remained unchanged.

The addition of Noviryn and Salicylic Acid to the nutrient medium during the second passage had a positive effect on the regeneration rate and induction of organogenesis *in vitro* (Fig. 11). It was found that in plants of the varieties Mezhyrichka 11 and Skarbnytsia the indicators of the studied traits increased compared to the control variant and were close to those obtained during the first passage.

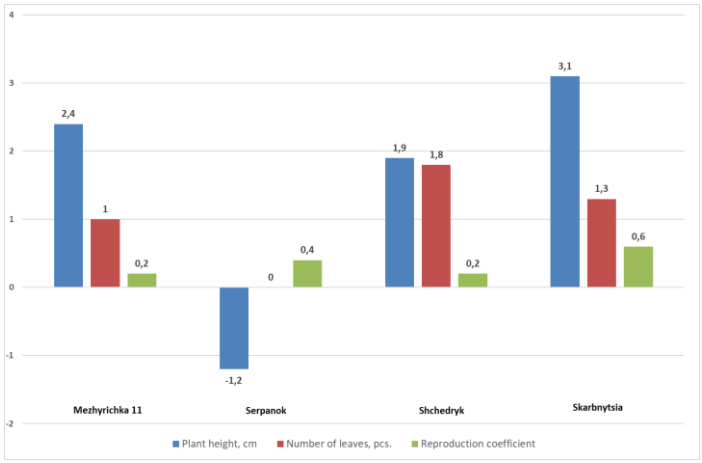


Figure 11 Effect of Noviryn and Salicylic Acid on the intensity of organogenesis *in vitro* culture of different potato varieties, second passage (+/- to the control)

In plants of Shchedryk variety, the intensity of growth and organ formation was higher than in the first passage. While during the first passage the height of plants increased by 0.4 cm, during the second passage it increased by 1.9 cm compared to the control variant. It should be added that, according to the results of long-term observations, this variety is well reproduced *in vitro*.

As a result of the studies, it was found that the survival of plants of different potato varieties *in vitro* depended on their genetic characteristics and the composition of the nutrient medium. Plants of the varieties Mezhyrichka 11, Serpanok and Shchedryk have a higher survival rate compared to the variety Skarbnytsia. On average, over two passages, the survival rate of Skarbnytsia plants was 93.8% compared to 98.6-99.4% for the other varieties. In general, Shchedryk variety is characterised by the highest plant survival *in vitro* (Table 3).

The introduction of Noviryn into the nutrient medium at a rate of 20 mg/100 ml of liquid in all varieties did not reduce, but even slightly increased the *in vitro* survival of the plants. On the contrary, the combined use of Noviryn and Dekasan in the norms (20 mg and 2.5 ml/100 ml of liquid), respectively, reduced the *in vitro* survival of the plants in all studied varieties. On average, regardless of the characteristics of the varieties, plant survival decreased from 98.5 to 95.5%. The largest drop in survival was observed in plants of Skarbnytsia variety (4.2%), and in Shchedryk variety it was the smallest and did not exceed 1.5% compared to the control variant (Table 3).

The simultaneous introduction of Noviryn and Salicylic Acid into the nutrient medium of Serpanok, Shchedryk and Skarbnytsia varieties reduced plant survival by only 1% compared to the control variant.

After the potato plants had been treated with chemotherapy elements *in vitro* culture, the virus infection content of the plants was investigated. According to the results of enzyme-linked immunosorbent assay (ELISA) testing, no virus antigens were detected. It should be noted that the goal of plant rehabilitation is not only to free microplants from viral infections, but also to increase the resistance of treated plants *in vivo* to secondary infection with viral diseases. The results of further studies indicate that during the growing season in the field, the first field generation from *in vitro* plants (category of pre-bred seed potatoes) proved to be resistant to secondary infection with virus diseases (Table 4).

Table 3 Restoration of plant regeneration *in vitro* culture using elements of chemotherapy

Variety (factor A)	Chemotherapy elements (factor B)	Plant survival, %	Average (factor A)	Average, (factor B)
Mezhyrichka 11	*Control*	99.0	98.8	98.5
	Control+ Noviryn	100.0		98.9
	Control+ Noviryn + Dekasan	97.0		95.5
	Control+ Noviryn + Salicylic Acid	99.0		97.8
Serpanok	*Control*	100.0	98.6	
	Control+ Noviryn	100.0		
	Control+ Noviryn + Dekasan	95.5		
	Control+ Noviryn + Salicylic Acid	99.0		
Shchedryk	*Control*	100.0	99.4	
	Control+ Noviryn	100.0		
	Control+ Noviryn + Dekasan	98.5		
	Control+ Noviryn + Salicylic Acid	99.0		
Skarbnytsia	*Control*	95.0	93.8	
	Control+ Noviryn	95.5		
	Control+ Noviryn + Dekasan	90.8		
	Control+ Noviryn + Salicylic Acid	94.0		

Table 4 Results of diagnostics of samples by enzyme-linked immunosorbent assay (ELISA)

Variety	Chemotherapy elements	Optical density of the enzymatic reaction product, optical units				
		PVY	PVM	PVS	PVX	PLRV
Mezhyrichka 11	*Control*	–	–	–	–	–
	Control+ Noviryn	–	–	–	–	–
	Control+ Noviryn + Dekasan	–	–	–	–	–
	Control+ Noviryn + Salicylic Acid	–	–	–	–	–
Serpanok	*Control*	–	0.688*	–	–	–
	Control+ Noviryn	–	–	–	–	–
	Control+ Noviryn + Dekasan	–	–	–	–	–
	Control+ Noviryn + Salicylic Acid	–	0.755	–	–	–
Shchedryk	*Control*	–	–	–	–	–
	Control+ Noviryn	–	–	–	–	–
	Control+ Noviryn + Dekasan	–	–	–	–	–
	Control+ Noviryn + Salicylic Acid	–	–	–	–	–
Skarbnytsia	*Control*	–	–	–	–	–
	Control+ Noviryn	–	–	–	–	–
	Control+ Noviryn + Dekasan	–	–	–	–	–
	Control+ Noviryn + Salicylic Acid	–	–	–	–	–

Legend: * up to 3000 optical units – severe infection;
up to 1500 optical units – medium infection;
up to 600 optical units – weak infection;
– no infection.

A weak infection with the mosaic leaf rolling virus (PVM) was found in Serpanok plants, cuttings of which were grown *in vitro* without the use of chemotherapy elements and with the use of Noviryn + Dekasan. But the level of infection is very low. In other variants, no traces of viral infections were found.

CONCLUSION

The analysis of the data obtained proves that the use of chemotherapy elements for the purpose of potato plants recovery *in vitro* culture not only helps to suppress the activity of viruses, but also leads to changes in morphological parameters and affects the rate of micropropagation. The influence of Noviryn, Dekasan and Salicylic Acid on the processes of regeneration and organogenesis of plants *in vitro* depended on the variety characteristics of potatoes.

Studies have shown that the use of Noviryn is the most effective element of chemotherapy for Serpanok and Skarbnytsia varieties. Its action stimulated an increase in plant height and the number of leaves per shoot, and increased the multiplication factor to 4.5 and 3.9, respectively. However, it is inappropriate to use this preparation for the improvement of the source material of Mezhyrichka 11.

Experimental data show that the use of Noviryn in combination with Dekasan is the best option for the recovery of plants of Mezhyrichka 11 variety, as it increases morphological parameters (+4.5 cm of stem length, +3.5 units of leaves) and the multiplication coefficient (+1.0).

The use of Noviryn in combination with Salicylic Acid had a positive effect on changes in the morphological parameters of all potato varieties under study. However, Shchedryk variety reacted negatively to stressful cultivation conditions, especially during the first passage, and requires additional research.

It should be noted that during the growing season in the field, the first field generation of *in vitro* plants was resistant to secondary infection with viral diseases.

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