Study of the Oxolin antiviral drug effect for the improvement of potato seed material during *in vitro* cultivation

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Abstract. The article presents an analysis of the potato breeding problems in Russia which are associated with the low phytosanitary state of potato seed material infected with viral and bacterial diseases, as a result, it is necessary to carry out its improvement and the use of Russian varieties. Experiments were conducted in the laboratory of plant microclonal propagation according to the methodological recommendations for improvement and accelerated propagation of seed potatoes. The results of research on refining the technology of seed potato improvement by adding the Oxolin antiviral drug to the standard nutrient medium MS; its optimal concentrations for obtaining the largest number of uninfected plants of the studied varieties were identified. For potato varieties Liga, Hussar, and Real, the optimal concentration was 20 mg/l of Oxolin, while in the varieties Aurora, Real, and Sirenevy tuman, the concentration was 30 mg/l. Therefore, it is necessary to search for antiviral drugs with an alternative mechanism of action which remains very relevant and practically significant. The research results obtained in this work can be applied both to optimize the cultivation regime of potato microclones *in vitro* and to enhance the productive process of the uninfected microplants.
Keywords: potato, meristem, improvement, Oxolin, *in vitro*, variety.

1. Introduction

Potatoes in Russia are on the same level as bread. In this regard, potato farming is considered the main branch of agriculture [1]. But since the last few years there have been significant changes in the ecology, potato management practice, and the importation of foreign varieties into the country contributes to the expansion of the area of viral diseases, increasing their harmfulness [2]. So more than 50% of the plantings of this crop are damaged by bacterial and viral diseases. Therefore, in order to obtain heavy, high-quality, and sustainable yields, it is necessary to use uninfected varieties of Russian breeding [3,4].

Currently, the main biotechnological method of improvement and accelerated reproduction of potato varieties is meristematic explant isolation followed by multiple cuttings of microplants grown *in vitro* under sterile conditions [5,6].

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According to scientists, this is the only effective way to obtain uninfected potato planting material, because the apical meristem of tuber sprouts is free from viral infection in most cases, and this method is non-competitive. But during the study of this issue, it was found that the effectiveness of potato improvement increases when combining the method of apical meristem with chemotherapy by adding antiviral drugs to the medium [4].

This raises the need to improve methods of improvement. When improving potatoes in *vitro* from harmful viruses, the effectiveness of various drug concentrations was determined, allowing to obtain a larger number of viable uninfected explants. Viruses cause significant damage to potato plantings and therefore varieties must be free of infection before they are transferred to the variety testing [7].

2. Materials and Method

Laboratory tests were carried out from 2020 to 2022 on the basis of the Velikie Luki State Agricultural Academy in the laboratory of microclonal reproduction. The research objects were mid-ripening potato varieties included in the State Register of Breeding Achievements, approved for use in the Russian Federation and recommended for cultivation in the North-West region. Apical meristem, regenerant plants, vegetative plants, minitubers, and tubers of the first and second field generations of the following potato varieties were used as the studied material: Hussar, Liga, Real, Sirenevy tuman, and Aurora [8,9].

The purpose of our research was to refine on the technology of improving seed potatoes by using Oxolin *in vitro* under the conditions of the North-Western region of the Russian Federation. The research objectives were to identify the Oxolin optimal concentrations in media for potato improvement *in vitro* [10].

In laboratory experiments, Oxolin was added in different concentrations to the medium for the explant improvement. **Oxolin** is an antiviral agent, white or white with a creamy or pinkish tinge, an odorless crystalline powder. It is freely soluble in water. It has a wide antiviral spectrum. It blocks the binding sites of the influenza virus with the cell membrane surface and protects cells from the virus penetrating. Active ingredient: Dioxotetrahydroxytetrahydronaphthalene (1,2,3,4-Tetrahydro-1,2,3,4-tetraoxonaphthalene dihydrate [11].

Design of laboratory experiments

Laboratory experiments were performed according to generally accepted methods.

Experiment 1

Study of the effect of different Oxolin concentrations on the yield of uninfected potato plants. Laboratory tests were carried out according to the design presented in the table below.

Table 1. Design of laboratory experiment No. 1

Variant	The medium composition for improvement
I (control)	Murashige and Skoog medium (MS)
II	Murashige and Skoog medium (MS) + Oxolin, 20 mg/l
III	Murashige and Skoog medium (MS) + Oxolin, 20 mg/l
IV	Murashige and Skoog medium (MS) + Oxolin, 40 mg/l

Each test variant had 20 test-tube plants. Triple analysis Oxolin was added to the medium during its preparation.

Laboratory procedure

Laboratory experiments on potato *in vitro* were conducted according to the "Methodological Recommendations for improvement and accelerated reproduction of seed potatoes" (1985).

The improving related to tissue culture was carried out by isolation of apical meristems – stem apical cones with 1-2 primordial leaf. For apex isolation, the buds of etiolated tuber sprouts were used, which were sterilized in sodium hypochlorite for 3-5 minutes and washed in distilled water. Apical meristems were isolated in a sterile laminar box under a binocular microscope MBS-2 at 16-32× magnification. Isolated apexes were immediately transferred to test tubes with Murashige and Skoog medium modified by Velikie Luki State Agricultural Academy. The medium was previously poured into test tubes, closed with cotton plugs and sterilized in an autoclave at a pressure of 0.1 MPa for 25 minutes.

Cuttings were carried out in a sterile laminar-box. Instruments (tweezers, scalpel) and Petri dishes were sterilized by burning on a alcohol lamp before cutting each plant. Regenerant plants were extracted with tweezers from test tubes and then cuttings. Single leaf cuttings were planted in test-tubes with medium to the depth of the internode.

The plants were cultivated *in vitro* at a temperature of 20-22°C, a relative humidity of 70-80%, illuminated with 3000-4000 lux fluorescent lamps with a 16-hour photoperiod. On the 7th day after planting, the processes of rhizo- and morphogenesis began, and on the 21st day, it was possible to re-cut fully grown plants. During this period, biometric data were recorded on days 7, 14, and 21: measuring plant height and root length, counting the number of internodes and roots.

To determine the infection with viruses, IEC and ELISA was used. Apical cuttings were used for this purpose [12].

In the experiment, 60 plants were studied in each variant.

3. Results

The main factors influencing the effectiveness of release of potato from viral diseases by apical meristem culture are the composition of medium, temperature, explant size, light, and varietal features [13].

Therefore, selecting optimal cultivation conditions for uninfected plants remains relevant.

The efficiency of potato improvement is increased when the apical meristem method is combined with chemotherapy by adding antiviral drugs to the medium [12].

In various sources, virazole, chitosan, interferon, lamivudine, cycloferon, etc. are proposed to be used as virus inhibitors.

The use of thermo- or chemotherapy allows to increase the size of meristem explants without loss of improvement efficiency, which increases the survival rate of explants on medium and as a result gives to obtain more uninfected regenerant plants [14].

In this paper, we studied the effect of different Oxolin concentrations on the yield of uninfected potato plants.

Table 2. Regeneration of potatoes from the meristem depending on the concentration of Oxolin in the medium, mg/l

Medium		**MS**	**MS + OK 20 mg/l**	**MS + OK 30 mg/l**	**MS + OK 40 mg/l**
Aurora	Number of regenerated plants, pcs.	47.0	43.0	41.3	35.7
	% of regenerated plants, pcs.	78.3	71.7	68.9	59.4
Hussar	Number of regenerated plants, pcs.	47.3	43.7	40.3	32.0
	% of regenerated plants, pcs.	78.9	72.8	67.2	53.3
Sirenevy tuman	Number of regenerated plants, pcs.	41.3	39.0	35.3	27.3
	% of regenerated plants, pcs.	68.8	65.0	58.9	45.5
Real	Number of regenerated plants, pcs.	41.7	42.0	38.3	33.7
	% of regenerated plants, pcs.	69.4	70.0	63.9	56.2
League	Number of regenerated plants, pcs.	46.3	45.3	41.3	32.7

	% of regenerated plants, pcs.	77.2	75.5	68.9	54.4

Oxolin has antiviral properties. The effect of different drug concentrations in the Murashige and Skoog medium was evaluated.

According to Table 2 (average for 2020-2022), Oxolin suppressed growth and negatively affected the survival rate of regenerated micro-plants of all studied varieties. There is a negative correlation of the antiviral drug concentration and the number of regenerated plants. At a maximum Oxolin concentration of 40 mg/l, the percentage of regenerated plants of all varieties was minimal. The highest yield of regenerated plants was obtained when cultivated on Oxolin-free Murashige and Skoog medium in Aurora and Hussar varieties.

The data in Table 3 indicate that Oxolin had a significant positive effect on the yield of uninfected potato plants. The grown source material was examined for the presence of occult pathogens by IEC and ELISA. At different stages of reproduction, viruses *A, S, L* were not detected, however, viruses *X, M* were encountered. No bacterial infection lesions were detected.

As a percentage, the maximum yield of uninfected plants was achieved in the variant MS + OK 40 mg/l for all varieties studied (97-100%). However, the high Oxolin concentration negatively affected the survival of meristems (Table 3). As a result, the yield of uninfected plants was lower than the control. In the example of the variety Aurora, on nutrient medium MS + OK 40 mg/l obtained 35.0 uninfected plants, and on standard Murashige and Skoog medium obtained 38.3 due to this, take root more explants (47 pcs, and on a medium with maximum Oxolin – 35.7 pcs).

Table 3 Regeneration of uninfected potato plants according to IEC

Medium		**MS**	**MS + OK 20 mg/l**	**MS + OK 30 mg/l**	**MS + OK 40 mg/l**
Aurora	Number of regenerated plants, pcs.	47.0	43.0	41.3	35.7
	Number of uninfected plants, pcs.	38.3	38.0	40.0	35.0
	% of uninfected plants	81.5	88.4	96.8	98.0
Hussar	Number of regenerated plants, pcs.	47.3	43.7	40.3	32.0
	Number of uninfected plants, pcs.	36.7	37.7	37.3	31.3
	% of uninfected plants	77.6	86.3	92.5	97.8
Sirenevy tuman	Number of regenerated plants, pcs.	41.3	39.0	35.3	27.3
	Number of uninfected plants, pcs.	32.3	34.0	34.7	27.0
	% of uninfected plants	78.2	87.2	98.3	98.9
Real	Number of regenerated plants, pcs.	41.7	42.0	38.3	33.7
	Number of uninfected plants, pcs.	30.0	37.3	37.3	32.7
	% of uninfected plants	71.9	88.8	97.4	97.0
League	Number of regenerated plants, pcs.	46.3	45.3	41.3	32.7
	Number of uninfected plants, pcs.	37.7	40.7	40.0	32.7
	% of uninfected plants	81.4	89.8	96.8	100.0

The work carried out showed that the optimal Oxolin concentration in the medium for improvement is 20 mg/l (for varieties Hussar, Real, and League) and 30 mg/l (for varieties Aurora and Sirenevy tuman). This makes it possible to obtain a larger number of uninfected plants, studied domestic varieties.

4. Discussion

The efficiency of seed production depends mainly on the seed material quality. The use of seed material affected by various pathogens is not economically profitable, production losses can be up to 80%. Studies on potato improvement we conducted earlier revealed that a combination of apical meristem techniques and chemotherapy should be used. It is advisable to add antiviral drugs to the standard medium which increases the yield of uninfected explants.

5. Conclusion

As a result of studies to refine the technology of seed potato improvement through the use of Oxolin *in vitro* under the conditions of the North-West region of Russia the optimal concentration of the antiviral drug was established. The greatest number of uninfected potato plants of varieties studied by us was obtained when using Oxolin in MS + OC 20 mg/l for varieties Liga — 40.7 pcs, Hussar — 37.7 pcs, and for variety Real — 37.3 pcs, and on medium with MS + OK 30 mg/l the greatest number of uninfected plants was obtained for variety Aurora 40,0 pcs, Real — 37.3 pcs, and Sirenevy tuman — 34.7 pcs. The data obtained analysis can be applied to enhance the productivity process of the uninfected micro-plants.

References

1. Yu.N. Fedorova, N.V. Lebedeva. Proceedings of the Velikie Luki State Agricultural Academy. **3**, P. 9-13 (2018)
2. V.I. Kulikova, V.P. Khodaeva, A. N. Gantimurova [et al.]. International Research Journal**.** **№1-2 (79)**, P. 14-18. (2019)
3. N.N. Semchuk, E.V. Oves, Ya.M. Abdushaeva, [et al.] Proceedings of the All-Russian Research-to-Practice Conference Veliky Novgorod, P. 368-373. (2021)
4. A.V. Korshunov, E.A. Simakov, Yu.N. Lysenko, B.V. Anisimov, A.V. Mityushkin, M.Yu. Gaitov. Actual problems and priority directions of innovative development of potato breeding. Dostizheniya nauki i tekhniki APK (Achievements of science and technology of agriculture). **32(3)**:12-20. (2018) DOI: https://doi.org/10.24411/0235-2451-2018-10303
5. Kh.Kh. Apshev, N.A. Timoshina, E.V. Knyazeva [et al.]. Kartofel i ovoshchi (Potatoes and vegetables), **1**, P. 30-32. (2019)
6. Savenkova I.V., Panferov V.G., Zherdev A.V., Dzantiev B.B. Proceedings of the International Forum “Biotechnology: state and prospects of development”, May 23-25, 2018, Moscow. P. 816-817.
7. E.V. Zhuravleva, S. V. Fursov. Kartofel i ovoshchi (Potatoes and vegetables). No**.5**, P. 6-9. (2018).
8. Antonova O. Yu., Apalikova O. V., Ukhatova Yu. V., Krylova E. A., Shuvalov O. Yu., Shuvalova A. R., Gavrilenko T. A. Improvement of micro-plants of three cultivated potato species (Solanum tuberosum L., S. phureja Juz. & Buk. and S. stenotomum Juz. & Buk.) from viruses by combined thermo-chemotherapy. Sel'skokhozyaistvennaya biologiya (Agricultural biology). **52(1)**:95-104. (2017) DOI: https://doi.org/10.15389/agrobiology.2017.1.95rus
9. E. A. Simakov, B. V. Anisimov, S. V. Zhevora [et al.]. Kartofel i ovoshchi (Potatoes and vegetables). No.**12**, P. 22-26, (2020)
10. The production process of original, elite, and reproductive seed potatoes. Practice guideline. Edited by A.M. Molko, B. V. Anisimov / FSBI “Rosselkhoznadzor”, FSBSI VNIIKH. Moscow, P.64 (2017)
11. B. V. Anisimov, S. N. Zebrin. Potato system. No.**4**, P. 36-41(2018)

12. Barsukova E. N., Kim I. V., Chekushkina T. N. Improvement and micro-reproduction in vitro of potato varieties for virus-free seed production. Dalnevostochny agrarny vestnik (Far Eastern Agrarian Bulletin). **(4(48)):**20-26. (2018) DOI: https://doi.org/10.24411/1999-6837-2018-14076
13. Dioxotetrahydroxytetrahydrophthalene (Dioxotetrahydrooxy-tetrahydronaphthalinum) [Oxolin]. – Text: electronic // Register of Medicines of Russia: [website]. – 2000-2022. – URL: https://www.rlsnet.ru/mnn_index_id_2657.htm (accessed: 05/17/2022). – Access mode: free.
14. A. I. Cheremisin, V. N. Kumpan. Vestnik Omskogo gosudarstvennogo agrarnogo universiteta (Bulletin of Omsk State Agrarian University). №**4(28)**. P. 87-91 (2017).