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THE INFLUENCE OF PHYSICAL CULTIVATION FACTORS ON MICROCLONAL REPRODUCTION OF SANITIZED IN THE CULTURE OF MERISTEMS *IN VITRO* PRIMARY MATERIAL OF POTATO

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INTRODUCTION

For a long time, seed growing of potato was based on the selection of healthy plants in general plantings. Clonal, bush, cluster, tuberous and a negative selection with the assessment of the material selected for the presence of viral infection were used.

A feature of modern seed growing of potato is the use for reproduction of the elite primary material, sanitized by methods of active treatment of contaminated potato varieties: by thermotherapy and meristem tissue culture, in combination with microclonal reproduction, which is intended to provide a disease-free primary material of potato in the volumes sufficient for the first links of seed growing[1,2,3].

By changing the environmental factors in the cultivation of plants *in vitro* under controlled conditions on artificial nutrient media, it is possible to regulate the process of organogenesis, in particular to induce the formation of tubers. This process is influenced by the varietal characteristics of plants: most varieties (95%) form micro tubers in 55-60 days, in others – the tuber formation lasts for a longer period. This process can be accelerated by the optimal interaction of the main factors that stimulate it: the content of carbohydrates and biologically active substances in the nutrient medium, the values of the photoperiod and temperature[4].

For the induction of microtubers in plants *in vitro*, a number of factors are used: mineral-enriched medium, higher sugar (4-8%) and kinetin (up to 1 mg/l) content, reduced photoperiod at a certain stage of plant growth, biologically active substances. The optimal ratio of these factors allows to obtain microtubers of full value with a diameter of 7-8 mm in 80-85% of planted culm segments.

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Taking into account the considerable cost of *in vitro*-derived seed material, the improvement of micro-reproduction techniques of the sanitized material is of particular relevance5. Therefore, a number of experiments were conducted at the Institute of Irrigated Agriculture of NAAS with the aim of developing new and improving existing ways and methods for the intensification of the process of microclonal reproduction of healthy primary material in the culture of meristems *in vitro*[6].

1. Tuberization of potato *in vitro* at different light intensities and cultivation temperatures

Light intensity and temperature are considered to be among the most important factors in the growing of microtubers *in vitro*. The Potato Biotechnology Laboratory of the Institute of Irrigated Agriculture of NAAS conducted a research considering the ratio of temperature and light intensity on the growth, development and productivity of plants of early-ripening potato variety Kobza[7]. Plants in vitro were grown at temperature conditions 14-16; 20-22; 24-26°C and lighting 500; 1500; 2000; 3000 lux (further lx).

The results of the studies showed a direct correlation of plant height in vitro with temperature. So, on the 20th day of observation, this index at a temperature of 24-26°C was higher, on average, by 2.7 cm than at a temperature of 14-16°C and by 0.5 cm than at 20– 22°C (Table 1).

On the day 40 of the observations, this dependence was maintained and the difference was 3.8 and 1.5 cm, respectively. Besides, *in vitro* plants grown at an illumination intensity of 1500 lx were 9.4% higher than in the illumination of 500 lx and 26.0 and 22.8%, respectively, than in 2000 and 3000 lx.

As the temperature of cultivation increased, the formation of stolons by plants was inhibited. On the 20th day of the observation, the number of plants that formed stolons at a temperature of 24-26°C, on average, was by 30.2 and 21.4 relative percent less than at a temperature of 14-16 and 20-22°C, respectively. Microtubers on the 20th day of the observation were formed at 7.3; 8.7 and 2.0% of plants, respectively, according to the growing temperatures of 14-16, 20-22 and 24-26°C. On the 40th day of the observations, the largest number of stolons, on average, was obtained at a temperature of 24-26°C – 63.6%, and microtubers – at 14-16°C – 69.5%.

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Table 1

The influence of temperature and light intensity on the growth and development of plants of early-ripening potato variety Kobza *in vitro*

Temperature, °C (A)	Light intensity, lx	Day of growing									
		20th				40th				60th	80th
		Plants height, cm	Number of internodes, pc.	% of plants that formed		Plants height, cm	Number of internodes, pc.	% of plants that formed			
				stolons	microtubers			stolons	microtubers	microtubers	microtubers
14-16	500	1.4	1.3	82.5	11.1	2.0	1.8	11.1	87.9	96.9	100.0
	1500	1.5	1.4	87.0	4.9	2.2	1.9	21.5	78.5	91.5	100.0
	2000	1.8	1.7	81.5	7.7	3.7	3.2	36.4	57.3	90.0	100.0
	3000	1.5	1.3	82.9	5.3	3.4	2.5	45.1	54.2	87.8	99.6
20-22	500	3.6	3.1	72.3	9.4	4.3	3.9	32.6	54.7	70.8	84.8
	1500	3.8	3.3	79.5	5.9	4.6	4.1	44.4	48.0	69.8	91.7
	2000	3.9	3.4	73.4	11.8	5.7	4.8	40.7	50.8	77.8	96.9
	3000	3.7	3.3	73.4	7.6	5.9	5.4	58.4	32.4	63.2	89.8
24-26	500	4.4	3.4	49.6	2.6	6.4	5.3	60.1	12.4	26.9	53.2
	1500	4.4	4.2	48.4	2.0	7.1	5.9	62.3	10.5	34.6	62.3
	2000	4.2	4.0	59.2	1.2	6.6	5.7	66.9	11.7	38.6	62.7
	3000	4.1	3.8	56.0	2.1	6.3	5.5	64.9	12.1	43.7	69.4

The correlation between the total number of microtubers that were formed by the plants *in vitro*, the output of microtubers with weight of more than 350 mg and the interaction of the studied factors is strong (R = 0.878 and 0.895). A strong inverse pair relationship was found between growing temperature and plant productivity *in vitro*: the number and mass of microtubers formed, the mass of the average microtuber. The pair correlation coefficients are, respectively, r=-0.895+0.141;-0.895±0.141;-0.801±0.189 (Table 2).

Table 2

Correlation coefficients (r) of the plant productivity in vitro of early-ripening potato variety Kobza depending on temperature and light intensity

Index	Temperature, °C, factor A	Light intensity, lx, factor B
The mass of the average microtuber, mg	-0.801±0.189	0.512±0.272
The mass of microtubers per plant, mg	-0.895±0.141	0.377±0.293
The output of microtubers with the mass of more than 350 mg, %	-0.673±0.234	0.590±0.255
The number of microtubers per plant, pc.	-0.895±0.141	0.089±0.315

There is a mean inverse correlation (r = – 0.673±0.234) between temperature conditions and the output of microtubers weighing more than 350 mg. On the 60th day of the observation, most microtubers were formed by the plants at a temperature of 14-16°C, 91.6% of their total number, at 20-22°C – 70.4% at 24-26°C with only 36.0%, and on the 80th day according to the specified temperature regimes, the index was 99.9; 90.8 and 61.9%, respectively.

On the day 20 ofgrowing, the light intensity had little effect on the induction of tuber formation and only 4.3-7.7% of the plants *in vitro* formed microtubers. The number of plants that formed stolons was also not affected by this factor during the mentioned period. On the 40th day of the observation, the highest quantity of the plants (51.7%) that formed microtubers were (on average, by factor) at a light intensity of 500 lx. On the 60th day of the observation, the number of microtuber-forming plants under illumination modes of 500, 1500, 2000, and 3000 lx, on average by factor, was 64.9; 65.3; 68.8 and 64.9%, respectively.

On the 80th day of the plant growing (Fig. 1), the percentage of microtubers was evened, on average by factor, a;most through all the variants of the experiment in exception of the variants with light intensity of 500 lx.

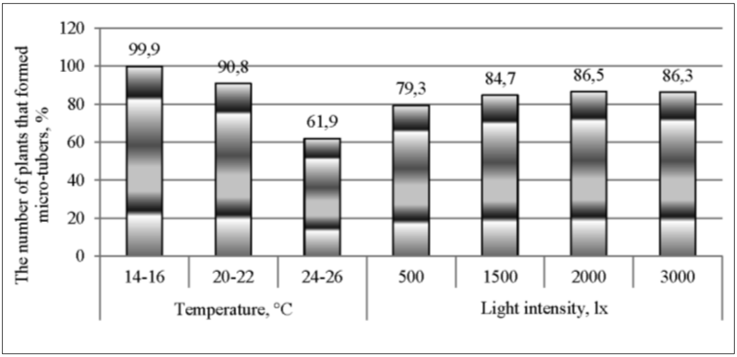

Fig. 1. Influence of temperature and light intensity on the processes of tuberization of the plants of early-ripening variety Kobza *in vitro*

This indicator was at light 1500-3000 lx 84.7-86.5%, and at 500 lx – 79.3%, i.e., the intensity of illumination almost did not affect the formation of microtubers by the plants *in vitro* of early-ripening potato variety Kobza.

Regarding the interaction of factors, it was found that the largest number of plants with microtubers was formed on the 80th day of growing at a temperature of 14-16°C and light intensity of 500-3000 lx – 99.6-100%. The multiple correlation index R was 0.878.

The interaction of growing temperature and light intensity had a very strong effect on the plant productivity *in vitro* (Table 3).

Table 3

Productivity of early-ripening potato variety Kobza plants *in vitro* depending on temperature and light intensity

Temperature, °C (A)	Light intensity, lx (B)	The mass of microtuber, mg	The mass of microtubers, mg/plant	The output of microtubers with the mass of more than 350 mg, %	The number of microtubers, pc./plant
14-16	500	163.8	224.6	6.8	1.2
	1500	210.9	266.4	15.8	1.2
	2000	263.8	369.1	24.3	1.1
	3000	262.0	363.7	20.9	1.2
20-22	500	95.0	82.3	0.4	0.9
	1500	138.8	143.0	6.0	1.1
	2000	140.7	154.1	6.2	1.1
	3000	174.0	186.1	11.4	0.9
24-26	500	60.5	42.8	0.0	0.6
	1500	128.9	81.4	6.8	0.6
	2000	132.1	82.4	7.2	0.6
	3000	132.2	119.1	9.4	0.8
The index of multiple correlation (R)		0.951	0.971	0.895	0.878
LSD_{05}, mg A		14.6	8.6		
B		12.1	13.9		

The average microtuber mass and the microtuber mass per plant were maximal at a temperature of 14-16°C and illumination of 2000 and 3000 lx and were, respectively, 263.8; 369.1 and 262.0; 363.7 mg. Also, in these variants, the highest yield of microtubers weighing 350 mg and more was observed – 24.3 and 20.9% (Fig. 2). The correlation between the mean microtuber mass, the microtuber mass per plant and the factors studied was very close: R = 0.951 and 0.971, respectively.

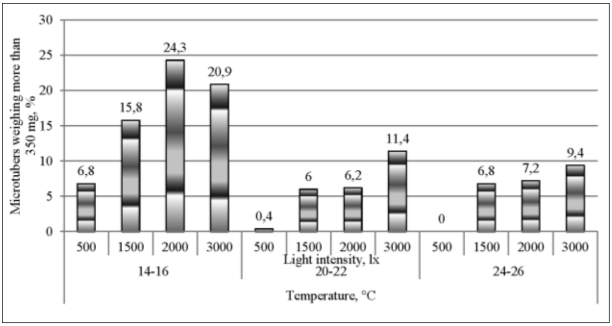

Fig. 2. *In vitro* productivity of early-ripening variety Kobza depending on temperature and light intensity

With the increase of growing temperature, the compensatory effect of the intensity of illumination on the performance of the plants in vitro is engaged: the total mass of microtubers per plant (r=0,377±0,293), the average mass of microtubers (r=0,512±0,272), and the output of microtubers weighing more than 350 mg (r=0,590±0,255). At a temperature of 20-22°C the productivity of plants was lower than at a temperature of 14-16°C. The lack of productivity is compensated at the highest level of illumination – 3000 lx: the mass of the average microtuber is increased by 83.2%, and the mass of microtubers per plant is increased 1.3 times in comparison to the illumination of 500 lx. At a temperature of 24-26°C, the highest productivity was obtained under the illumination of 3000 lx.

By the results of ANOVA, on average for 3 years, the greatest effect on the mass of microtubers per plant had the temperature regime (factor A), much less – the illumination (factor B) (Fig. 3).

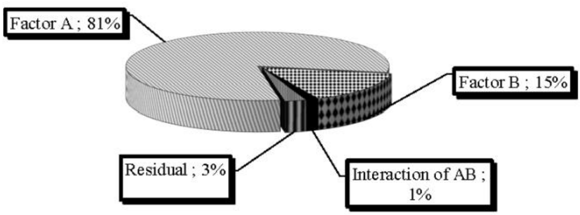

Fig. 3. The share of the effect of temperature and light intensity on the formation of microtuber mass per plant *in vitro* of early-ripening potato variety Kobza

The regression analysis of the obtained data allowed to obtain linear mathematical models of the dependence of productivity of the potato plants of early-ripening variety Kobza *in vitro* depending on the interaction of growing temperature and light intensity (Table 4).

Table 4

Regression equation of the dependence of productivity indexes of the potato plants of early-ripening variety Kobza *in vitro* on temperature (X_1) and light intensity (X_2)

Index	Equation
The mass of average microtuber, mg	$Y = 321.4 – 11.45X_1 + 0.0334X_2$
The mass of microtubers per plant, mg	$Y = 541.16 – 22.85X_1 + 0.0439X_2$
The output of microtubers with the mass of more than 350 mg, %	$Y = 24.0 – 1.17X_1 + 0.00466X_2$
The number of microtubers per plant, pc.	$Y = 1.88 – 0.0507 X_1 + 2.3E – 5X_2$

It is determined that the decisive factor in the process of plant morphogenesis *in vitro* of the plants of early-ripening potato variety Kobza and the formation of their productivity are growing temperature conditions, light intensity affects much less. The maximum performance of the plants *in vitro* was obtained through the usage of a temperature regime of 14-16°C and illumination of 2000-3000 lx.

At the growing temperature of 14-16ºC, the cost of a unit of product was 5.21 UAH, and its increase to 20-22 and 24-26ºC caused the increase of the cost of microtuber 1.2 and 1.9 times and the decrease of profitability – by 54 and 147%, respectively (Table 5).

Light intensity had no significant impact on the economic indexes. The lowest cost of a microtuber and the maximum profitability of production are formed at a growing temperature of 14-16ºC and light intensity of 500 lx: UAH 4.93 and 224%, respectively.

In the conditions of southern arid climate, it is important to select microtubers with the possibly largest mass, which in the future will allow obtaining the maximum number of healthy potato minitubers and will help to increase the yields of pre-basic and basic seeds. Taking this into account during determination of the optimal elements of the technology of growing microtubers of early-ripening potato variety Kobza in vitro, it was established that the optimal productivity and economic indexes of growing laboratory plants are at a growing temperature of 14-16ºC and light intensity of 3000 lx. Herewith, the number of microtubers per plant was 1.2 pc., the average microtuber mass was 262.0 mg, the microtubers mass per plant was 363.7 mg, and the number of microtubers with the mass of 350.0 mg and more was 20.9%; the production cost of a microtuber was 5.31 UAH at the profitability of 201%.

Table 5

Economic efficiency of growing microtubers of early-ripening potato variety Kobza *in vitro* depending on temperature and light intensity

Temperature, °C (A)	Light intesity, lx (B)	The number of microtubers per plant, pc.	The expenditures per plant, UAH	The cost of a microtuber, UAH	The conditional net profit, UAH/tuber	Profitability, %
14-16	500	1.2	5.92	4.93	11.07	224
	1500	1.2	6.01	5.01	10.99	219
	2000	1.1	6.15	5.59	10.41	186
	3000	1.2	6.37	5.31	10.69	201
20-22	500	0.9	5.97	6.63	9.37	141
	1500	1.1	6.28	5.71	10.29	180
	2000	1.1	6.41	5.83	10.17	174
	3000	0.9	6.55	7.28	8.72	121
24-26	500	0.6	6.11	10.18	5.82	57
	1500	0.6	6.43	10.72	5.28	49
	2000	0.6	6.62	11.03	4.97	45
	3000	0.8	6.70	8.38	7.62	91

2. The intensity of potato tuberization *in vitro* depending on the duration of the photoperiod, temperature, level of nitrogen nutrition and the period of substitution of the nutrient medium

A four-factor experiment was conducted by microclonal laboratory of the Institute of Irrigated Agriculture of NAAS to determine the most optimal regime of the middle-ripening potato variety Nevska tuberization *in vitro*. The following factors were put to the study: A – duration of photoperiod (10 and 16 hours); B – temperature regimes (18-20 and 23-25ºC); C – nitrogen content in the nutrient medium (full and half norm, no nitrogen); D – medium substitution times (on the 10th and 20th day).

Cuttings of the plants of the middle-ripening potato variety Nevska were grown on a complete liquid nutrient medium of Murashige, Skoog (MS) at 10 and 16 hours of illumination per day at temperatures of 18-20 and 23-25ºC. On the 10th day, the cuttings of one group were transferred from a complete nutrient solution with a nitrogen content of 868 mg/L to the solution with ½ nitrogen (434 mg/L) and with no nitrogen. In the second group of the plants the nutrient medium was changed after 20 days. The photoperiod and temperatures were maintained as before.

Observations on the plant growth and tuberization intensity showed that *in vitro* plants were 10.8% higher under the repacement of the nutrient medium on the 10th day of growing at the background of 10-hour illumination than those exposed to 16-hour illumination (Table 6). There was no difference in the number of internodes. The number of plants with microtubers on the twentieth day of the observation at thethe background of the ten-hour photoperiod was 1.7%, and at the sixteenth hour – 0.5%. But on the 40th day, this index was, respectively, 17.9 and 11.3%, and on the 60th – 39.4 and 39.1%. The total number of microtubers during the entire vegetation period was 83.4% at the ten-hour photoperiod, and 73.9% at the sixteenth hour.

Table 6

Influence of growing conditions on growth, in vitro development of the plants of the middle-early potato variety Nevska at changing the medium on the 10th day of cultivation

Photoperiod, hours	**Temperature, °C**	**Nitrogen content**	**The indexes on the day of cutting**									**Number of plants with microtubers, %**
			20th			40th			60th			
			Plants height, cm	**Number of internodes, pc.**	**Number of plants with microtubers, %**	**Plants height, cm**	**Number of internodes, pc.**	**Number of plants with microtubers, %**	**Plants height, cm**	**Number of internodes, pc.**	**Number of plants with microtubers, %**	
10	18-20	Full norm	3.6	3.2	5.5	5.0	4.5	43.0	5.5	5.8	71.9	95.0
		½ norm	3.7	2.9	1.3	4.4	3.8	21.2	4.7	4.1	42.5	94.5
		No nitrogen	3.5	3.0	1.7	4.2	3.6	25.4	4.7	4.3	45.4	98.1
	23-25	Full norm	4.7	3.7	0.0	6.0	4.8	5.8	6.6	5.8	18.4	52.1
		½ norm	4.2	3.1	0.7	5.9	4.1	7.5	6.8	5.4	30.5	72.5
		No nitrogen	4.6	3.5	0.7	5.4	4.2	4.2	6.7	5.7	27.5	88.1
16	18-20	Full norm	3.5	3.2	1.2	5.4	4.9	24.3	6.1	5.8	47.5	92.6
		½ norm	3.1	3.0	0.5	3.7	3.6	13.6	4.4	4.1	40.6	87.5
		No nitrogen	2.8	2.6	0.8	3.3	3.3	14.2	3.6	3.7	38.7	97.0
	23-25	Full norm	4.6	3.8	0.0	5.9	5.4	5.8	6.5	6.2	37.9	46.4
		½ norm	4.2	3.7	0.3	5.5	4.7	7.8	6.2	5.6	23.5	54.2
		No nitrogen	4.2	3.5	0.0	5.1	4.2	2.0	5.9	5.2	9.4	65.9

It was found that the increase in the temperature of growing to 23-25ºC provided the increase in the number of internodes of the plants *in vitro* during the first 20 days of growth and development by 19.0%, on the 60th and 80th day – by 15.6 and 29.9%, respectively. Plants height under the maintaining of the temperature regime at the level of 23-25ºC was 30.0-33.4% higher during the whole cultivation period than at 18-20ºC. On the 40th day of cultivation at the temperature of 18-20°C the number of plants with microtubers was 23.6% that was 4.3 times higher than at the temperature of 23-25°C. In general, for the entire cultivation period, at 18-20°C the microtubers were formed on 94.1% of the plants in vitro and at 23-25°C – on 63.2%.

It was determined that replacing of the nutrient medium on the 20th day of cultivation resulted in more intensive process of tuberization than at replacing it on the 10th day (Table 7). Overall, 82.8% and 78.7% of the plants formed microtubers, respectively.

Table 7

Influence of growing conditions on growth, in vitro development of the plants of the middle-early potato variety Nevska at changing the medium on the 20th day of cultivation

Photoperiod, hours	**Temperature, °C**	**Nitrogen content**	**The indexes on the day of cutting**									**Number of plants with microtubers, %**
			20th			40th			60th			
			Plants height, cm	**Number of internodes, pc.**	**Number of plants with microtubers, %**	**Plants height, cm**	**Number of internodes, pc.**	**Number of plants with microtubers, %**	**Plants height, cm**	**Number of internodes, pc.**	**Number of plants with microtubers, %**	
10	18-20	Full norm	4.1	3.4	4.4	5.5	5.0	39.4	6.2	5.6	73.0	94.3
		½ norm	3.5	3.0	3.4	4.3	4.0	37.1	5.1	4.9	71.5	98.7
		No nitrogen	3.1	2.8	0.8	4.0	3.5	27.8	4.4	4.5	75.4	98.3
	23-25	Full norm	4.7	3.9	0.0	6.2	5.2	5.6	6.9	6.4	12.1	52.1
		½ norm	4.9	3.8	0.0	6.9	5.2	4.7	7.5	6.0	37.7	79.6
		No nitrogen	5.2	4.2	0.0	6.7	5.6	2.5	7.6	6.8	25.1	87.6
16	18-20	Full norm	3.9	3.6	1.1	6.0	5.4	16.1	6.2	6.0	52.1	92.7
		½ norm	4.2	3.8	0.0	5.9	5.4	10.9	6.1	5.6	56.1	100.0
		No nitrogen	4.0	3.6	0.0	5.5	5.0	10.6	6.1	5.7	53.7	99.2
	23-25	Full norm	4.4	3.7	0.0	6.1	5.6	4.4	6.7	6.3	26.2	46.5
		½ norm	4.8	3.7	0.0	6.1	5.3	4.8	6.6	6.0	38.7	69.4
		No nitrogen	4.8	4.1	0.0	5.9	5.3	6.0	6.4	5.7	39.9	75.3

At transferring of the plants in vitro on the day 10 from full nutrient medium to medium containing ½ amount of nitrogen and medium without nitrogen, on the 40th day of cultivation the plants height decreased by 12.6 and 19.3% and the number of internodes – by 17,3 and 21.9%, respectively. On the 60th day of cultivation, the plants height and the number of internodes in the medium with a half norm of nitrogen were lower by 10.5 and 18.6%, respectively, than at the background with the full norm, and in the medium without nitrogen – by 15.4 and 19.9%, respectively. However, without the use of nitrogen and at the application of the half of its norm for the whole period of cultivation, the total number of plants with microtubers increased by 15.8 and 5.7 percent, respectively. When transferring the plants in vitro on the 20th day of cultivation, these rates were 18.7 and 15.5 percent, respectively.

There was a strong correlation dependence between the number of generated microtubers per plant and the interaction of the studied factors (R = 0.863) (Table 8).

Table 8

Coefficients (r) of correlation dependence of the plants productivity *in vitro* of the middle-early potato variety Nevska in regard to the duration of the photoperiod, temperature, level of nitrogen nutrition and substitution period of the nutrient medium

Studied factors	**Mass of average microtuber, mg**	**Mass of microtubers per plant, mg**	**Number of microtubers per plant, pc.**
Photoperiod, hours, factor A	0.365±0.198	0.184±0.210	-0.097±0.212
Temperature, °C, factor B	-0.818±0.123	-0.852±0.112	-0.830±0.119
Content of nitrogen in the nutrient medium, mg/L, factor C	0.034±0.213	0.038±0.213	0.055±0.213
Substitution period, days, factor D	0.283±0.204	0.288±0.204	0.212±0.208

The temperature conditions of cultivation have a greater influence (pair correlation coefficient r=-0.830±0.119) than the photoperiod (r=-0.097±0.212), the terms of substitution of the nutrient medium (r=0.212±0.208) and the nitrogen content in it (r=0.055±0.213).

The analysis of the data shows that at sixteen-hour illumination in comparison with the ten-hour one, on average, the mass of the micro-tuber increased by 17.8%, and the mass of tubers per plant – by 19.8% (Table 9).

Table 9

Influence of the level of nitrogen nutrition, temperatures, photoperiod duration and the time of substitution of the nutrient medium on the productivity of potatoes of the middle-early variety Nevska *in vitro*

Temperature, °C	**Photoperiod, hours**	**Substitution of the medium, day**	**Nitrogen content**	**Mass of microtuber, mg**	**Mass of microtubers, mg/plant**	**Number of microtubers, pc./plant**
18-20	10	10	Full	172.7	163.9	0.9
			½ norm	139.0	133.7	1.0
			No	160.3	153.8	1.0
		20	Full	172.7	163.9	0.9
			½ norm	204.8	211.8	1.0
			No	201.3	213.7	1.1
	16	10	Full	226.7	227.0	1.0
			½ norm	183.4	152.9	0.8
			No	179.9	174.5	1.0
		20	Full	226.7	227.0	1.0
			½ norm	247.6	283.2	1.2
			No	259.0	286.4	1.1
23-25	10	10	Full	81.5	48.9	0.5
			½ norm	89.6	60.0	0.7
			No	101.8	88.8	0.8
		20	Full	81.5	48.9	0.5
			½ norm	108.4	86.2	0.8
			No	128.8	115.0	0.9
	16	10	Full	111.1	52.8	0.5
			½ norm	139.5	71.4	0.5
			No	107.5	69.4	0.6
		20	Full	111.1	52.8	0.5
			½ norm	153.2	99.9	0.7
			No	148.6	111.3	0.7
Multiple correlation index (R)				0.94	0.918	0.863
LSD_{05}, mg			A	11.3	9.7	
			B	10.9	8.8	
			C	8.2	9.0	
			D	7.7	6.0	

A very strong correlation was found between the mass of the average microtuber (R = 0.94), the mass of microtubers per plant (R = 0.918) and the studied factors. At 18-20°C temperature, the average microtuber mass was 68.8% higher than in the 23-25°C regime, and the microtubers mass per plant was 2.5 times higher. This is confirmed by the strong inverse pair relationship between the temperature and plant productivity, i.e., with the increase of cultivation temperature, the mass of the average microtuber (r=-0.818±0.123), the mass of microtubers per plant (r=0.852±0.112) and the number of microtubers formed decrease (r =-0.830±0.119).

At transferring of the cultivated plants from the nutrient medium with a full nitrogen rate to the medium with a half of the norm the mass of the average microtuber increased by 6.9%, the mass of microtubers per plant– by 11.6%, and in the medium without nitrogen, these indexes were higher by 8.7% and 23.1%, respectively.

The mass of an average microtuber and the microtubers mass per plant were higher at the replacing of the nutrient medium on the day 20 than on the day 10 by 31.9 and 49.7%, respectively. So, 20 days of cultivation completely satisfied the requirements in nitrogen, and the subsequent vegetation, the presence of this element did not affect the process of tuberization.

Regression analysis of the gathered data allowed obtaining linear mathematical models of the potato plants productivity of the middle-early ripening variety Nevska in vitro depending on the level of nitrogen nutrition, growing temperature, duration of photoperiod and term of substitution of the nutrient medium (Table 10).

Calculations of the economic efficiency of growing microtubers of middle-early variety of potato Nevska in vitro, depending on the studied factors, showed that the production cost per microtuber, on average, at the cultivation temperatures of 18-20^{o}C was 1.8 times lower than at 23– 25°C.

Table 10

Equations of regression depending on the productivity indexes of the plants *in vitro* of middle-early potato variety Nevska at different temperature of growing (X_1), photoperiod (X_2), term of the nutrient medium substitution (X_3) and level of nitrogen supply (X_4)

Index	Equation
Mass of average microtuber, mg	$Y = 374.62-16.86X1 + 6.28X_2 + 2.92X_3 + 0.0198X_4$
Mass of microtubers per plant, mg	$Y = 524.58-24.77X_1 + 4.44X_2 + 4.19X_3 + 3.3E\text{-}5X_4$
Number of microtubers per plant, pc.	$Y = 2.23-0.0717X_1-0.00694X_2 + 0.00917X_3 + +0.000132X_4$

The production cost of microtuber at the use of a sixteen-hour photoperiod increased by 13.2%, compared to ten hours of illumination, on average by the factor (Table 11). substitution of the nutrient medium on the 20th day of cultivation reduced the production cost by 8.7%; owing to the transfer of the cultivated plants from a nutrient medium with a full nitrogen rate to a medium with a half of the norm, the production cost of a microtuber is reduced by 17.6%, for a medium without nitrogen – by 25.7%.

The maximum productivity and the outpay of investments at the determination of the optimum elements of technology for growing microtubers of the middle-early potato variety Nevska *in vitro*, taking into account such a significant factor as the size of the obtained microtubers, was got under the combination of the factors: 16 hours illumination, 18-20°C cultivation temperature, the substitution of the nutrient medium on the 20th day of cultivation on the medium with a half of the norm of nitrogen. Herewith, the number of microtubers per plant is 1.2 pc., the mass of an average microtuber is 247.6 mg, the microtuber mass per plant is 283.2 mg; the production cost of a microtuber is 5.24 UAH at the profitability of production at 205%.

Table 11

Economic efficiency of potato microtubers production of the middle-early variety Nevska in vitro depending on the cultivation temperature, photoperiod, nutrient medium substitution term and nitrogen supply level

Temperature, °C	Photoperiod, hours	The term of substitution of the nutrient medium, day	Nitrogen content	Number of microtubers per plant, pc.	Expenditures per plant, UAH	Microtuber production cost, UAH	Conditional pure profit, UAH/microtuber	Profitability, %
18-20	10	10	Full norm	0.9	6.00	6.67	9.33	140
			½ norm	1.0	5.95	5.95	10.05	169
			No nitrogen	1.0	5.90	5.90	10.10	171
		20	Full norm	0.9	6.12	6.80	9.20	135
			½ norm	1.0	6.07	6.07	9.93	164
			No nitrogen	1.1	6.02	5.47	10.53	192
	16	10	Full norm	1.0	6.22	6.22	9.78	157
			½ norm	0.8	6.17	7.71	8.29	107
			No nitrogen	1.0	6.12	6.12	9.88	161
		20	Full norm	1.0	6.34	6.34	9.66	152
			½ norm	1.2	6.29	5.24	10.76	205
			No nitrogen	1.1	6.24	5.67	10.33	182
23-25	10	10	Full norm	0.5	6.60	13.20	2.80	21
			½ norm	0.7	6.55	9.35	6.65	71
			No nitrogen	0.8	6.49	8.11	7.89	97
		20	Full norm	0.5	6.73	13.46	2.54	19
			½ norm	0.8	6.68	8.35	7.65	92
			No nitrogen	0.9	6.62	7.36	8.64	117
	16	10	Full norm	0.5	6.84	13.68	2.32	17
			½ norm	0.5	6.79	13.57	2.43	18
			No nitrogen	0.6	6.73	11.22	4.78	43
		20	Full norm	0.5	6.97	13.95	2.05	15
			½ norm	0.7	6.92	9.88	6.12	62
			No nitrogen	0.7	6.86	9.81	6.19	63

CONCLUSIONS

1. A strong and average inverse pair relationship between the temperature and the plant productivity during *in vitro* cultivation of early-ripening potato variety Kobza (*r = -0.801; -0.895; -0.673; -0.895*) has been established, i.e., by increasing the cultivation temperature, the mass of the average microtuber decreases, the mass of the microtubers per plant decreases, the output of microtubers weighing more than 350 mg and the number of microtubers formed decreases, respectively.

2. It was found that the decisive factor in the process of the plant morphogenesis *in vitro* of the early-ripening potato variety Kobza and the formation of its productivity were the cultivation temperature conditions, and the light intensity affected much less.

3. In the conditions of southern arid climate, it is of particular importance to obtain *in vitro* microtubers with bigger mass, which have an increased capacity to produce the maximum number of healthy potato minitubers that further will be favorable for getting high yields of pre-basic and basic seed material. Therefore, taking into account this factor, at the determination of the optimum elements of growing technology of the early-ripening potato variety Kobza microtubers in the meristem *in vitro* culture, it was determined that optimal productivity indexes and economic efficiency of the in vitro plants cultivation are provided at the temperature of 14-16ºC and light intensity 3000 lx. The number of microtubers per plant – 1.2 pc., the mass of an average microtuber – 262.0 mg, the mass of the microtubers per plant – 363.7 mg, the number of microtubers weighing more than 350.0 mg – 20.9%; production cost of the microtuber – 5.31 UAH at the profitability of 201%.

4. The maximum productivity and outpay of the investments at the determination of the optimum elements of growing technology of microtubers of the middle-ripening potato variety Nevska *in vitro* culture was obtained at the combination of the factors: illumination of 16 hours, cultivation temperature 18-20^{o}C, substitution of the full nutrient medium Murashige, Skoog (MS) on the medium with a half of the norm of nitrogen on the 20th day of the cultivation. The number of microtubers per plant – 1.2 pc., the mass of an average microtuber – 247.6 mg, the mass of the microtubers per plant – 283.2 mg; the production cost of the microtuber – 5.24 UAH at the profitability of production of 205%.

SUMMARY

Taking under consideration a considerable cost of the seed material obtained *in vitro*, the development and improvement of new techniques and methods for intensifying of the process of microclonal reproduction of sanitized potato primary seed material is of a particular relevance. It is established that the maximum productivity and outpay of the investments is provided by the cultivation of the early-ripening potato variety Kobza at the

temperature of 14-16ºC and the light intensity of 3000 lx; of the middle-early potato variety Nevska: with the illumination of 16 hours, the cultivation temperature of 18-200C, the substitution of the full nutrient medium Murashige, Skoog (MS) on the 20th day of the cultivation on the medium with a half of the norm of nitrogen. The number of microtubers per plant is 1.2 and 1.2 pc., the mass of an average microtuber is 262.0 and 247.6 mg, and the microtubers mass per plant is 363.7 and 283.2 mg, respectively.

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