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FORMATION OF POTATO MICROTUBERS *IN VITRO* DEPENDING ON TEMPERATURE AND LIGHT INTENSITY

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Aim. To specify optimal techniques influencing the increase of intensity of potato tuber formation in meristem culture *in vitro*. M**ethods.** Integrated use of laboratory, mathematical and statistical, calculation and comparison methods and the method of systematic analysis. **Results.** The paper represents experimental data about the influence of temperature and light intensity on the induction of tuber formation under microclonal reproduction of the health-improved initial material. It proves that the decisive factor in the process of morphogenesis of plants *in vitro* of the early maturing potato variety Kobza and their productivity is temperature conditions for cultivation. **Conclusions.** Optimal indexes of productivity and economic efficiency are maintained by growing test-tube plants under the temperature of cultivation of 14–16 ºC and the light intensity of 3,000 lux. The number of microtubers per plant was 1.2 pieces, the weight of an average microtuber was 262,0 mg, the weight of microtubers per plant was 363,7 mg, the number of microtubers weighing more than 350,0 mg was 20,9 %; the cost price of microtubers was 5,31 UAH with the profitability of 201 %.

Keywords: potato**,** microtubers, temperature mode, light intensity, *in vitro*.

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INTRODUCTION

A recent decade has been remarkable for intense development of various biotechnologies, the most relevant one being the elaboration of efficient measures to fight viral diseases in potato growing [1–3]. In particular, the techniques of health-improving potatoes using meristem culture have been developed; productive ways of cultivation and clonal microreproduction of plants as well as highly sensitive immunological [4–6] and PCR-methods of virus diagnostics are widely used. There is active search for the ways of creating virus-resistant varieties using gene engineering [7], cell and tissue technologies [8, 9].

The specificity of modern potato cultivation is the restoration of elite varieties using the initial material, health-improved via the methods of active treatment of infected potato varieties, including thermotherapy and the culture of meristem tissue in combination with microclonal reproduction, which should ensure obtaining the initial material of potatoes, free from diseases and in the volume, sufficient for the needs of primary units of seed production.

The changes in the environmental factors during the *in vitro* cultivation of plants in the controlled conditions in artificial media may be used to regulate the organogenesis process, in particular, to induce the tuber formation. This process is affected by variety specificities of plants: in most varieties (95 %) microtubers are formed within 55–60 days, in others, it takes more time. This process may be accelerated using optimal interaction of the main stimulating factors: the content of carbohydrates and biologically active substances in the medium, the values of photoperiod and temperature, *etc.* [10–12].

At the initial stages of usage, the seed material, health-improved using the biotechnology methods, is notable for better quality, as during its production the synthesis of the viral protein in plants is delayed and, as a result, the tempo of viral infection accumulation is slowed down. At the same time, taking into consideration considerable cost of the seed material, obtained *in vitro*, the improvement of microreproduction of the health-improved material is of special relevance. Light intensity and temperature are considered some of the most important factors while growing microtubers *in vitro*.

The aim of this work was to determine optimal technological ways, affecting the increase in the intensity of potato tuber formation in meristem culture *in vitro*.

MATERIALS AND METHODS

The experiment, using common methods [11–13], was conducted in conditions of microclonal laboratory to identify the most optimal mode of tuber formation in the culture *in vitro* for Kobza potato variety. Two factors were studied: temperature mode (14–16, 20–22, 24–26 °C) and light intensity (500, 1500, 2000, 3000 lux).

RESULTS AND DISCUSSION

The analysis of the data obtained about the temperature mode testified to direct dependence of the *in vitro* plant height on temperature. For instance, on day 20 of the observations, this index at the temperature of 24–26 °C was 2.7 cm higher on average compared to the temperature of 14–16 °C, and 0.5 cm higher compared to the temperature of 20–22 °C (Table 1). On day 40 of the observations this dependence was the same and the difference was 3.8 and 1.5 cm, respectively. Here the plants *in vitro*, grown at light intensity of 1,500 lux, were 9.4 % taller than those at light intensity of 500 lux, and 26.0 and 22.8 % higher compared to light intensity of 2,000 and 3,000 lux.

The increase in the cultivation temperature led to the inhibition of the process of stolon formation by plants. On day 20 of the observations, the number of plants which formed stolons at the temperature of 24–26 °C was on average 30.2 and 21.4 relative per cent less compared to the temperature of 14–16 and 20–22 °C, respectively. On day 20 of the observations, the microtubers were formed in 7.3, 8.7 and 2.0 % of plants at the cultivation temperature of 14–16, 20–22 and 24–26 °C, respectively. On day 40 of the observations, the highest average number of stolons was obtained at the temperature of 24–26 °C (63.6 %), and that of microtubers – at 14–16 °C (69.5 %).

The correlation dependence between the total number of microtubers, formed by plants *in vitro*, the yield

Table 1. The impact of temperature and light intensity on the growth and development of the plants of the early maturing variety Kobza in culture *in vitro*

T, °C	Light intensity, lux	Day of cultivation									
		20				40				60	80
		Height of plants, cm	Number of internodes	% of plants which formed		Height of plants, cm	Number of internodes	% of plants which formed		% of plants which 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

of microtubers with the weight of over 350 mg and the interaction of the investigated factors is significant (R = 0.878 and 0.895). A significant reverse dual interrelation between the temperature of cultivation and the productivity of the plant *in vitro* was observed: the number and weight of formed microtubers and the weight of the average microtuber. The dual coefficients of the correlation are, respectively, $r = -0.895 \pm 0.141$; -0.895 ± 0.141; -0.801 ± 0.189.

The average reverse correlation dependence was observed between the temperature conditions and the yield of microtubers with the weight over 350 mg ($r = -0.673 \pm 0.234$). On day 60 of the observations, most microtubers were formed by the plants at the temperature of 14−16 °C – 91.6 % from their total amount, at 20−22 °C − 70.4 %, at 24−26 °C − only 36.0 %, and on day 80, according to the mentioned temperature modes this index was 99.9, 90.8 and 61.9 %.

On day 20 of the cultivation, the light intensity practically did not affect the induction of tuber formation: only 4.3−7.7 % of plants *in vitro* formed the microtubers. During the mentioned period this factor did not affect the number of plants which formed the stolons either. On day 40 of the observations, most plants (51.7 %) which formed the microtubers, were on average at light intensity of 500 lux. On day 60 of the observations, the number of such plants at the light modes of 500, 1,500, 2,000 and 3,000 lux was 64.9, 65.3, 68.8 and 64.9 % on average respectively.

On day 80 of growing plants, the ratio of microtubers, formed by them was almost even for all the experiment variants except for the variants with light intensity of 500 lux. The mentioned index at the light intensity of 1,500–3,000 lux was 84.7–86.5 % and at 500 lux – 79.3 %, that is the light intensity practically did not affect the formation of microtubers by the *in vitro* plants of the early maturing variety Kobza.

As for the interaction of the affecting factors of temperature and light intensity, it was determined that the highest number of plants with microtubers were formed on day 80 of the cultivation at the temperature of 14−16 °C and light intensity of 500−3000 lux – 99.6−100 %. The multiple correlation index was R = 0.878. The interaction of the temperature of cultivation and light intensity had great effect on the productivity of plants *in vitro* (Table 2).

The weight of the average microtuber and the weight of microtubers per plant were maximal at the tem-

Table 2. The productivity of the plants of the early maturing potato variety Kobza in the culture *in vitro* depending on temperature and light intensity

T, °C	Light intensity, lux	Weight of a microtuber, mg	Weight of microtubers, mg/plant	Yield of microtubers with the weight over 350 mg, %	Number of microtubers per one 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
Multiple correlation index (R)		0.951	0.971	0.895	0.878
HIP_{05} for temperature factor		14.6	8.6	–	–
HIP_{05} for light intensity factor		12.1	13.9	–	–

perature of 14−16 °C and light intensity of 2,000 and 3,000 lux and were 263.8, 369.1 and 262.0, 363.7 mg, respectively. Also the highest yield of microtubers with the weight of 350 mg and higher was observed in these variants – 24.3 and 20.9 %. The correlation dependence between the weight of the average microtuber, the weight of microtubers per plant and the investigated factors was very close: R = 0.951 and 0.971, respectively.

When the temperature of cultivation is increased, there comes an additional factor of the compensatory effect of light intensity on the productivity indices of the plants *in vitro*: the total weight of microtubers per plant (r = 0.377 ± 0.293), the average weight of a microtuber (r = 0.512 ± 0.272) and the yield of microtubers with the weight of over 350 mg (r = 0.590 ± 0.255). At the temperature of 20−22 C the productivity of plants was lower than at the temperature of 14−16 °C. The lack of productivity is compensated at the highest level of light intensity – 3,000 lux: the weight of an average microtuber is increased by 83.2 %, and the weight of microtubers per one plant − 1.3-fold compared to the light intensity of 500 lux. At the temperature of 24−26 °C the highest productivity was obtained at the light intensity of 3,000 lux.

The regression analysis of the obtained data allowed building linear mathematical models of the dependence of the productivity of the plants of the early maturing variety Kobza in the culture *in vitro* on the interaction of the cultivation temperature and light intensity.

The equations of the regression of the dependence:

the weight of the average microtuber *in vitro (Y)* on the temperature (X_1) and light intensity (X_2) − $Y = 321.4 – 11.45X_1 + 0.0334X_2$;

the weight of microtubers per one plant *(Y)* on the temperature (X_1) and light intensity (X_2) − $Y = 541.16 – 22.85X_1 + 0.0439X_2$;

the yield of microtubers with the weight over 350 mg *(Y)* on the temperature (X_1) and light intensity (X_2) − $Y = 24.0 – 1.17X_1 + 0.00466X_2$.

It was determined that the decisive factor in the morphogenesis process of plants *in vitro* of the early maturing potato variety Kobza and the formation of their productivity is the temperature mode of cultivation, as light intensity is of much lesser effect. The maximal indices of plant productivity *in vitro* were obtained using the temperature mode of 14−16 °C and light intensity of 2,000−3,000 lux.

At the temperature of cultivation of 14−16 ºC the cost of one production unit was UAH 5.21, and the increase in temperature to 20−22 and 24−26 ºC caused the 1.2-fold and 1.9-fold increase in the cost price of a microtuber and the decrease in economic efficiency by 54 and 147 %, respectively (Table 3).

Table 3. The economic efficiency of the cultivation of potato microtubers of the early maturing potato variety Kobza in the culture *in vitro* depending on temperature and light intensity

T, °C	Light intensity, lux	Number of microtubers per one plant	Expenses per one plant, UAH	Cost price, UAH/ microtuber	Operating profit, UAH/ microtuber	Economic efficiency, %
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

Light intensity does not have a considerable effect on economic indices. The lowest cost price of the microtuber and the maximal economic efficiency of the production were achieved at the temperature of cultivation of 14–16 ºС and light intensity of 500 lux: UAH 4.93 and 224 %, respectively.

CONCLUSIONS

In conditions of dry southern climate, it is important to select microtubers with the highest weight, which will allow obtaining the maximal amount of health-improved potato minitubers and promote the increase in the yield of prebasic and basic seeds. While determining the optimal elements of the cultivation technology for the microtubers of the early maturing potato variety Kobza in the culture *in vitro*, it was defined that the cultivation of test-tube plants at the temperature mode of 14–16 ºС and light intensity of 3,000 lux ensured better indices of productivity and economic efficiency. Here the number of microtubers per one plant was 1.2 it., the weight of the average microtuber – 262.0 mg, the weight of microtubers per one plant – 363.7 mg, the number of microtubers with the weight over 350.0 mg – 20.9 %; the cost price of a microtuber – UAH 5.31 with the economic efficiency of 201 %.

Формування мікробульб картоплі в культурі *in vitro* залежно від температури та інтенсивності освітленості

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Мета. Визначити оптимальні технологічні прийоми, що впливають на підвищення інтенсивності бульбоутворення картоплі в культурі меристем *in vitro*. **Методи.** Комплексне використання лабораторного, математико-статистичного, розрахунково-порівняльного методів та системного аналізу. **Результати.** Наведено експериментальні дані щодо впливу температури та інтенсивності освітленості на індукцію бульбоутворення за мікроклонального розмноження оздоровленого вихідного матеріалу. Встановлено, що вирішальним фактором у процесі морфогенезу рослин *in vitro* ранньостиглого сорту картоплі Кобза та формуванні їх продуктивності є температурні умови культивування. **Висновки.** Оптимальні показники продуктивності та економічної ефективності забезпечує вирощування пробіркових рослин за температури культивування 14–16 ºС та інтенсивності освітленості 3000 лк. При цьому кількість мікробульб на одну рослину становить 1,2 шт., маса середньої мікробульби – 262,0 мг, маса мікробульб на одну рослину – 363,7 мг, кількість мікробульб масою понад 350,0 мг – 20,9 %; собівартість мікробульби – 5,31 грн при рентабельності 201 %.

Ключові слова: картопля, мікробульби, температур-ний режим, інтенсивність освітлення, *in vitro.*

Формирование микроклубней картофеля *in vitro* в зависимости от температуры и интенсивности освещения

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Цель. Определить оптимальные технологические приемы, влияющие на повышение интенсивности клубнеобразования картофеля в культуре меристем *in vitro*. **Методы.** Комплексное использование лабораторного, математико-статистического, расчетно-сравнительного методов и системного анализа. **Результаты.** Приведены экспериментальные данные по влиянию температуры и интенсивности освещения на индукцию клубнеобразования при микроклональном размножении оздоровленного исходного материала. Установлено, что решающим фактором в процессе морфогенеза растений *in vitro* раннеспелого сорта картофеля Кобза и формировании их продуктивности являются температурные условия культивирования. **Выводы.** Оптимальные показатели продуктивности и экономической эффективности обеспечивает выращивание пробирочных растений при температуре культивирования 14–16 ºС и интенсивности освещения 3000 лк. При этом количество микроклубней на одно растение составляет 1,2 шт., масса среднего микроклубня – 262,0 мг, масса микроклубней на одно растение – 363,7 мг, количество микроклубней массой свыше 350,0 мг – 20,9 %; себестоимость микроклубня – 5,31 грн при рентабельности 201 %.

Ключевые слова: картофель, микроклубни, температурный режим, интенсивность освещения, *in vitro*.

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