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Influence of density of saccharose and aucsins upon the process of formation of tubers of potato in crop of stolons in vitro

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The purpose. To determine the optimum technological methods influencing heightening intensity of tuber-formation of potato of early ripening variety Kobza in crop of stolons *in vitro*. **Methods.** Complex use of laboratory, mathematical-statistical, calculation-comparative methods and system analysis. **Results.** Experimental data are resulted on influence of density of saccharose and aucsins in nutrient medium on induction of tuber-formation at reproduction of potato in crop of stolons *in vitro.* **Conclusions.** The maximum productivity and reward payment of holdings at determination of optimum elements of technique of growing of microtubers of early ripening variety of potato Kobza in crop of stolons *in vitro* is generated at adding in nutrient medium of saccharose in dose of 80 g/l and kinetin in dose of 0,5 mg/l. Mass of average microtuber is 53,2 mg, amount of stolons which have organized microtubers — 87% at the cost price of microtuber 2,49 hrn and profitableness of production 141%.

Key words: *crop of stolons in vitro, tuber-formation, amount of stolons, height of plants, saccharose, kinetin, nutrient medium, mass of microtubers.*

Biotechnologies are widely developing in various directions, effective measures have been developed to combat against viral diseases in the seed production of potatoes [1-4]. The methods of potato recovery using meristem culture, productive methods of cultivation and clonal micropropagation of plants, highly sensitive immunological [5-7] and PCR methods for the diagnosis of viruses were recommended. The search for ways to create virus-like varieties using genetic engineering [8], cellular and tissue technologies [9-12].

The process of tuber formation *in vitro* can be regulated by a number of endo-and exogenous factors (excess of assimilants, hormonal status of plants, temperature regime, photoperiod, light intensity, pH and culture medium composition), which is the basis for obtaining high-quality material in the primary seed production of potato. It significantly improves plant growth *in vitro* by adding growth regulators to the culture medium. These include auxins, cytokinins, gibberellins, abscisic acid, ethylene. Of particular importance for the cultivation of plants *in vitro* are the first three groups of substances.

The group of cytokinins includes adenine, kinetin, BAP, zeatin, etc. Synthesis of cytokinins in plants occurs mainly in the roots. An increase in the intensity of tuber formation in the cultivation of plants on a culture medium with a content of cytokinins in particular kinetin has been established, which activates the incorporation of amino acids into the polypeptide chain, and this results in more intensive protein biosynthesis and enhanced cell division. Of particular importance in this case is the concentration of phytohormone in the culture medium [13]. From the concentration and the ratio of biologically active substances in the plant depends the beginning and intensity of stolono- and tuber formation. The same pattern is also characteristic of biologically active substances used by plants from the culture medium.

For the best development of plants *in vitro*, sucrose should be used as a source of carbohydrate nutrition in the culture medium. The concentration of sucrose is a factor of stolon- and tuber formation and accumulation of microtuber mass.

Research objective: Study of the influence of the concentration of sucrose and phytohormones on potato tuber process of the early-ripening variety of potatoes Kobza in culture *in vitro* stolons to increase the amount of production of the original healthy planting stock.

Materials and methods of research. In the conditions of a microclonal laboratory, we conducted an experiment in which two factors were put to study: factor A - sucrose concentration (6, 8 and 16%), factor B - kinetin content (0.5, 1.0 and 2.5 mg/L).

On the 20th day of cultivation, from the cuttings of plants *in vitro*, potato sort Kobza, which were grown on a culture medium Murashige, Skoog (MS) with a sugar content of 6% and kinetin 0.5 mg/L, were removed stolons and transplanted on to a culture medium which consists of sugar 6, 8 and 16% and kinetin - 0.5; 1.0 and 2.5 mg/L. Cultivation of microtubers was carried out at a temperature of 20-22°C in diffuse illumination.

The studies were performed according to generally accepted methodologies. In order to obtain the initial potato plants that were improved by the biotechnological method *in vitro*, a thermo-chemotherapy method was used in combination with the culture of the apical meristems according to the "Methodological recommendations for conducting studies with potatoes" [14], the methodological recommendations "Improvement of potatoes in in vitro culture" [15], "Optimizing the methods of healing, multiplying and protecting potato seeds from a viral infection" [16] and "Biotechnological methods for obtaining and evaluating potato recovery" [17]. The experiments were carried out according to standard methods [18]. The economic efficiency of production of a healthy source material in an *in vitro* culture was calculated based on the actual cost of microtubers according to the technological maps.

Research results. Observations show that the content of sucrose and kinetin significantly influenced the height of the plants. So, already on the 20th day after the transplantation, at a content of 60 and 80 g/L of sucrose, the plant height, on average by factor, was 3.2; 2.7 cm, while using 160 g/L, only 0.8 cm. The number of stolons that formed the microtuber was higher when using 80 g/L of sucrose and amounted, on average by factor, to 35.6%, which exceeded other variants by 12.4 and 11.2% (Table 1). At the same time, a large number of stolons that formed sprout were formed with application of 60 g/L sucrose and on average exceeded other variants by 11.1 and 43.3%.

On the 40th day of cultivation, the situation with plant height remained unchanged. At the same time, the number of stolons that formed the microtubers increased at a sucrose concentration of 60 g/L and amounted on average by factor of 57.8%, exceeding the other experiment variants by 8.9 and 6.7%.

1. Effect of concentration sucrose and phytohormones on potato tuber process in culture *in vitro* stolons of early-ripening potato sort Kobza

Concentration of sucrose,% (A)	Kinetin content, mg/L (B)	The indicators of plant growth and development per day,					Average microtuber mass, mg	Mass of microtubers on 1 stolone, mg	Number of stolons that formed the microtuber %
		20th			40th				
		Height of sprouts, cm	Number of stolons that formed the microtubers,%	Number of stolons that formed sprouts,%	Height of sprouts, cm	Number of stolons that formed the microtubers,%			
6	0,5	3,6	23,0	86,6	3,7	73,3	48,8	42,1	80
	1,0	3,2	26,7	66,7	3,3	53,3	41,5	24,9	60
	2,5	2,9	20,0	70,0	2,9	46,7	51,3	25,7	50
8	0,5	1,6	46,7	56,7	2,5	56,7	53,2	42,5	87
	1,0	3,3	33,3	73,3	4,2	46,7	61,0	42,7	70
	2,5	3,1	26,7	60,0	3,2	43,3	49,2	32,8	60

16	0,5	0,95	13,3	40,0	1,2	50,0	48,8	29,3	60
	1,0	0,9	36,7	30,0	0,9	60,0	31,1	19,7	63
	2,5	0,45	23,3	23,3	0,6	43,3	35,2	17,6	50
Multiple correlation index (R)							0,623	0,761	0,813
LSD_{05} A							4,8	4,2	
B							5,9	5,3	

By the amount of kinetin it should be noted that this factor also influenced the indicators of the accumulation of productivity by plants. So, on the 20th day of cultivation with application of 1.0 mg/L kinetin the plant height, on average by factor, was higher in comparison with the concentration of 0.5 and 2.5 mg/L by 13.6 and 19.0%, according. At the concentration of kinetin in the nutrient solution of 1.0 mg/L, the number of stolons that formed the microtuber was high and amounted to 32.2% on average, which exceeded the other variants by 4.5 and 8.9%. The number of stolons that formed sprouts depending on the concentration of kinetin 0.5; 1.0 and 2.5 mg/L was accordingly 61.1; 56.7 and 51.1%.

On the 40th day of cultivation, the plant height, depending on the kinetin content in the culture medium, remained unchanged, but the number of sprouts that formed the microtuber was high, at a kinetin concentration of 0.5 mg/L by 6.7 and 15.6%.

At the end of vegetation, the average correlation of the mass of microtubers (R=0.623) and the strong one for microtubers per 1 stolon (R=0.761) and the number of stolons that formed the microtuber (R=0.813) from the interaction of the factors that were studied (Table 2).

1. Coefficients (r) of the correlation of economic efficiency and productivity in culture *in vitro* stolons early ripening sort of potato Kobza on the concentration of sucrose and phytohormones

Index	Concentration of sucrose, %, factor A	Content of kinetin, mg/L, factor B
Average microtuber mass, mg	-0,597±0,138	-0,178±0,169
Mass of microtubers on 1 stolone, mg	-0,576±0,140	-0,498±0,149
Number of stolons that formed the microtubers,%	-0,339±0,161	-0,738±0,116
Prime coast, UAH/microtuber	0,606±0,136	0,702±0,122
Rentability, %	-0,564±0,142	-0,677±0,126

On average by factor, the mass of the average microtuber was the highest with 60 and 80 g/L sucrose and amounted to 47.2 and 54.5 mg, which exceeded the variants using a concentration of 160 g/L at 22.9 and 41.9%, according. The mass of microtubers per 1 stolon was the highest (39.3 mg) with 80 g/L sucrose, which is higher than in other variants by 8.4 and 17.1 mg. It should be noted the average reverse pairing relationship between the concentration of sucrose in the culture medium and the productivity of plants, that is, with an increase in the content of sucrose, the weight of the average microtuber decreases (r=-0.597 + 0.138), the mass of the microtuber per stolon (r=-0.576 + 0.140) and number of stolons that formed the microtubers (r=-0.339 + 0.161).

Regarding the content of phytohormone in the culture medium, it should be noted that the concentration of kinetin also influences the formation of plant productivity in culture *in vitro* stolons of the early-ripening sort of potato Kobza. In particular, strong feedback was found between the concentration of kinetin and the number of stolons that formed microtubers (r=-0.738 + 0.116). With the decrease in the kinetin content to 0.5 mg/L in the culture medium, their amount increases by 11.4 and 22.4%, compared to a concentration of 1.0 and 2.5 mg/L, respectively. Somewhat less influence is exerted by kinetin on mass formation of microtubers on one stolon (r=-0.498 + 0.148), it was greater with 0.5 mg/L of kinetin

and exceeded other variants by 8.9 and 12.6 mg. The concentration of kinetin in the culture medium had practically no effect on the accumulation of mass of microtubers (r=-0.178 + 0.169).

By the end of the growing season, the maximum number of stolons that the microtubers formed was, on average, by factors, with 80 g/L of sucrose and 0.5 mg/L of kinetin (Figure 1).

Regression analysis of the obtained data made it possible to obtain linear mathematical models of the dependence of the productivity of potato plants of the early sort Kobza variety in culture *in vitro* stolons on the sucrose concentration and kinetin content in the culture medium (Table 3).

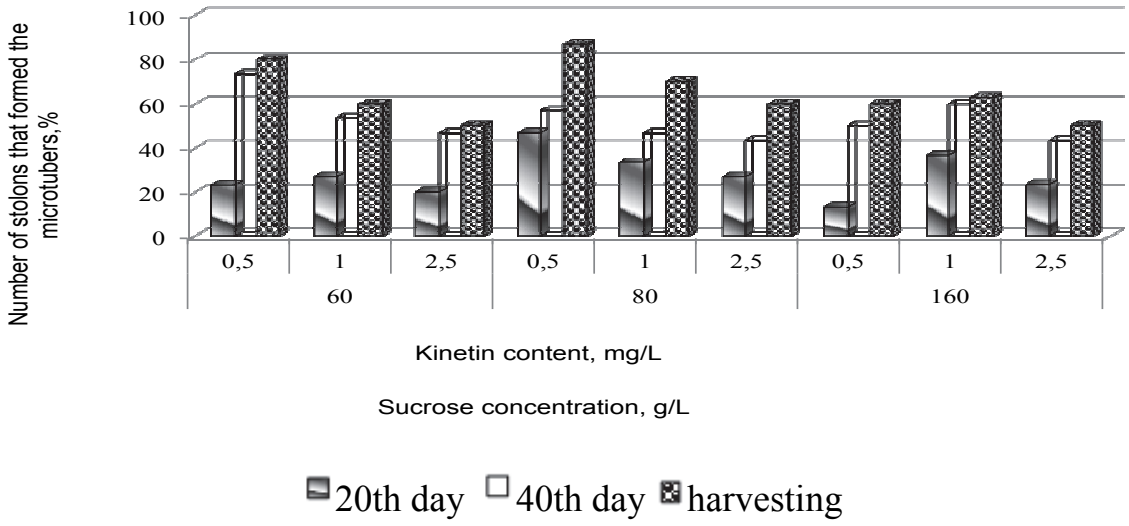

Fig. 1. Potato tuber process in culture in vitro stolons of the early ripening sort of potato Kobza depending on the concentration of sucrose and the content of kinetin in a culture medium

1. The regression equation for the dependence of potato tuber process in culture *in vitro* stolons of the early ripening sort of potato Kobza on the concentration of sucrose (X_1) and the content of kinetin in the culture medium (X_2)

Index	Form of equation
Average microtuber mass, mg	$Y = 61,17 – 1,21X_1 – 1,83X_2$
Mass of microtubers on stolone, mg	$Y = 50,37 – 1,23X_1 – 5,42X_2$
Number of stolons that formed the microtubers,%	$Y = 87,44 – 0,93 X_1 – 10,28X_2$

Calculations of the economic efficiency of cultivation of microtubers of the early sort of potato Kobza in culture *in vitro* stolons, depending on the factors studied, showed that the cost of one microtuber, on average by factor, when using a culture medium containing 8% sucrose by 10.2 and 30.3% lower, than at 6 and 16%, respectively; With a kinetin content of 0.5 mg/L - by 13.2 and 32.2% lower than when using 1.0 and 2.5 mg/L, respectively (Table 4). The same dependence was also noted in terms of profitability.

2. Economic efficiency of cultivation of microtubers of early-ripening sort of potato Kobza in culture *in vitro* stolons depending on sucrose concentration and kinetin content in culture medium

Sucrose concentration, % (A)	Kinetin content, mg/L (B)	Number of microtubers per plant, pcs	Costs per plant, UAH	Prime coast, UAH/microtuber	Conditional net income, UAH/micrituber	Rentability, %
6	0,5	0,80	2,08	2,60	3,40	131
	1,0	0,60	2,11	3,52	2,48	71
	2,5	0,50	2,24	4,48	1,52	34
8	0,5	0,87	2,17	2,49	3,51	141
	1,0	0,70	2,20	3,14	2,86	91
	2,5	0,60	2,32	3,87	2,13	55
16	0,5	0,60	2,53	4,22	1,78	42
	1,0	0,63	2,56	4,06	1,94	48
	2,5	0,50	2,68	5,36	0,64	12

Conclusions

Maximum productivity and efficiency of investment in determining the optimal cell technology growing microtubers early maturing varieties of potatoes in the culture Kobza stolons formed in vitro when added to the culture medium of 80 g/L of sucrose and 0.5 mg/L of kinetin: average weight of microtubers - 53.2 mg, the number of stolons, microtubers formed a - 87.0% at a cost of microtubers - 2.49 UAH and rentability 141%.

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