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Influence of storey of cuttings of test glass plants and nutrient medium upon induction of formation of tubers of potato in vitro of grades of different groups of ripeness

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The purpose. To determine optimum regime for formation of tubers of grades of potato in vitro of different groups of ripeness depending on storey of cuttings of test glass plants and content of nutrient medium. **Methods.** Complex use of laboratory, mathematical-and-statistical, calculation-and-comparative methods and systems analysis. **Results.** Experimental data about influence of storey of cuttings of test glass plants and content of nutrient medium on induction of formation of tubers are resulted at reproduction of different by group of ripeness grades of potato in crop in vitro. **Conclusions.** The best productivity indexes are gained at growing microtubers of middle-ripening grade of potato Yavir on nutrient medium of Institute of irrigation farming agriculture of NAAS at plants from 1– 3-rd storey of a cutting. So, the mass of average microtuber has made 502 mg, mass of microtubers for 1 plant — 509,8 mg. Yield of microtubers in mass more than 350 mg — 84,7%, intensity of formation of tubers made 101,5%.

Key words: *potato, grade, crop in vitro, storey of a cutting, microtuber, productivity.*

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Introduction. The main problem of potato growing in the south of Ukraine is the increased threat of the defeat of plants by viral, fungal and bacterial diseases, greatly complicates the management of seed production, reduces the quantity and quality of the crop [1-4], so the modern seed production of potatoes in this region, referred to as the zone of risky farming, is impossible without the improvement of existing methods of reproducing the healthy initial seed material in *in vitro* conditions.

Analysis of recent research and publications. The effectiveness of the biotechnological method depends on a number of factors: the intensity of the illumination, the temperature regime, the duration of the photoperiod, and others [5-9].

The basis of plant tissue cell culturing in *in vitro* condition is the composition of the nutrient medium, which includes micro- and macro salts, vitamins, stimulants, etc. [6, 8, 9-13]. Studies [14] show that even the plants parts which are spliting during the cutting are distinguished by the influence on the induction of microtuber formation and on the resistance of the plants against the viral infection [15]. It should be noted that various cultivars have their reaction to the same conditions of cultivation in *in vitro* conditions [16-20]. In addition, the complex influence of the differend tiers of steems, potato cultivars of different ripening groups and the composition of the nutrient medium is not well-studied, which means that their complex research acquires an urgency in solving the problem of optimizing the process of tuber formation in *in vitro* culture.

The purpose of research. To determine the most optimal regimes of tuber formation of potato cultivars of different ripening groups depending of the parts of plants *in vitro* and the composition of the nutrient medium.

Materials and methods of research. The studies were carried out under microclonal laboratory conditions. Three factors were put into the study: factor A – cultivars of different ripening groups (early - Tiras, mid-early - Levada and mid-ripening cultivar Yavir), factor B - nutrient media with different

compositions: Murashige, Skoog (MS); modified by Institute of Potato NAAS (IP NAAS); modified by the Institute of Irrigated Agriculture NAAS (IIA NAAS) (table 1), factor C was represented by various parts of plants (1-3 and 4-6 tiers of stem).

Table 1. The composition of nutrient media that are being studied, mg/l.

Main Ingredients	Murashige, Skoog (original)	Modified nutrient media	
		IP NAAS	IIA NAAS
Macrosalts:			
NH_4NO_3	1650,000	1250,000	1650,000
KNO_3	1900,000	1100,000	1900,000
$Ca(NO_3)_2x4H_2O$	-	440,000	440,000
KH_2PO_4	170,000	970,000	170,000
Na_2ЭДТА*	37,300	37,300	37,300
$FeSO_4x7H_2O$	27,800	27,800	27,800
$MgSO_4x7H_2O$	370,000	770,000	370,000
$CaCl_2x2H_2O$	440,000	-	-
Microsalts:			
$Na_2MoO_4x2H_2O$	0,250	0,250	0,250
H_3BO_3	6,200	6,200	6,200
$MnSO_4x4H_2O$	22,300	22,300	22,300
$ZnSO_4x4H_2O$	8,600	8,600	8,600
KI	0,830	0,830	0,830
$CuSO_4x5H_2O$	0,025	0,025	0,025
$CoCl_2x6H_2O$	0,025	0,025	0,025
Vitamins:			
Nicotinic acid	0,500	-	-
Pyridoxine (B_6)	0,500	1,000	1,000
Thiamine (B_1)	0,100	1,600	1,600
Ascorbic acid	-	3,000	3,000
Mesoinositol	100,000	-	
Growth regulators:			
Kinetin	0,010	0,500	0,500
Adenine	-	-	-
Gibberlic acid	1,000	-	-
Heteroauxin	2,000	1,000	1,000
Other substances:			
Agar-agar	10000,000	-	
Sucrose	30000,000	-	-
Sugar	-	70000,000	70000,000
Casein hydrolyzate	1000,000	-	-

At the 40th and 60th days of cultivars measured the number of microtubers generated, the harvesting of which was carried out on the 80th day of cultivation with the determination of the mass of the average microtube, the mass of microtubers per plant, the yield of microtubers weighing more than 350 mg, and the intensity of tuber formation.

The biotechnological method on the basis of thermo-, chemotherapy in combination with the apical meristem culture *in vitro* was used to obtain the healthy initial seed material. All operations were

conducted in accordance with the "Methodological recommendations for research on potatoes" [21], the methodological recommendations "Improvement of potato in *in vitro* culture" [22], "Optimization recovery, reproduction and protection potato seeds from viral Infection" [23] and "Biotechnological methods for obtaining and assessing the improvement of potatoes" [24]. Experiments were carried out according to generally accepted methods [25].

Results of research. On the 40th day, the highest intensity of tuber formation was recorded at the Yavir cultivar on the IP NAAS nutrient medium - 78.8% versus 14.5% and 43.5%, when grown on MS and IIA NAAS nutrient media, respectively (table 2).

Table 2. Induction of microtubers formation, depending of the nutrient medium, various parts of potato plants *in vitro* and cultivars of different ripening groups

Cultivar	Nutrient media	Tires of stem	% of the plants that formed microtubers on day		
			40th	60th	80th
Tiras	MS	1-3	0,0	2,0	5,5
		4-6	0,0	0,0	3,0
	IP NAAS	1-3	53,0	63,5	80,5
		4-6	49,5	49,5	51,5
	IIA NAAS	1-3	53,0	61,5	71,5
		4-6	45,5	47,5	59,5
Levada	MS	1-3	31,5	44,5	47,0
		4-6	12,5	18,0	23,5
	IP NAAS	1-3	67,5	69,5	70,5
		4-6	49,0	49,0	49,0
	IIA NAAS	1-3	60,0	72,0	82,0
		4-6	75,5	77,5	77,5
Yavir	MS	1-3	18,5	61,5	87,5
		4-6	10,5	49,5	81,0
	IP NAAS	1-3	74,5	89,5	100,5
		4-6	83,0	88,5	97,5
	IIA NAAS	1-3	46,0	90,0	101,5
		4-6	41,0	77,5	97,5
LSD_{05}	partial differences	A	6,9	7,1	10,9
		B	13,2	11,0	17,9
		C	10,5	9,6	11,3
	main effects	A	2,8	2,9	4,4
		B	5,4	4,5	7,3
		C	3,5	3,2	3,8

The early-ripening cultivar Tiras on the MS nutrient medium did not form a microtubers, but growing on the nutrient media of the IP NAAS and IIA NAAS show 51.3% and 49.3% of microtuber formation, respectively. The highest intensity of tuber formation of the Levada cultivar was noted when growing on nutrient medium modified by the IIA NAAS - 67.8% versus 21.8% and 58.3% on the nutrient media of MS and IP NAAS, respectively. On average, 50.5% of microtubers were formed on 1-3 tires of stems on all the studied cultivars, and 40.7% on tiers 4-6.

On the 60th day of observations, tuber formation of Tiras cultivar in percentage terms on IP NAAS and IAA NAAS nutrient media is 56.5 and 54.5%, against, when grown on MS nutrient medium - 1.0%. Levada cultivar show the highest rate of tuber formation only on the nutrient medium modified by

IIA NAAS - 74.8% versus 31.3 and 59.3% on nutrient media MS and IP NAAS, respectively. The mid-ripening cultivar Yavir showed the best indices of tuber formation in comparison with other cultivars: 55.5; 89.0 and 83.8% (MS, IP NAAS and IIA NAAS, respectively). Comparing the intensity of tuber formation of different cultivars depending of the tires of stems, it was noted that at 1-3 tiers the plants formed 61.6% of microtubers, which is 10.8% higher than on tiers 4-6.

On the 80th day, the tuber formation rate of early-ripening cultivar Tiras on the MS nutrient medium was 4.3%, against the same indicator on the nutrient medium modified by the IP NAAS, 66.0%, that on 0.5% more than growing on IIA NAAS nutrient medium. The best indicator of productivity of the Levada cultivar was obtained on the nutrient medium of IIA NAAS - 79.8% against 35.3% and 59.8% on the MS and IP NAAS nuutrient media, respectively. The mid-ripening Yavir was show 99.5%; 99,0 and 84,3% of microtubers formation (IIA NAAS, IP NAAS and MS), respectively. Most microtubers were formed when using plants from 1-3 tiers of stems - 71.8% against 60.0% from tiers 4-6.

When analyzing the interaction of the nutrient medium and the potato cultivars, it can be seen that the early-ripening cultivar Tiras has the highest mass of the average microtuber and the weight of microtubers per one plant on the nutrient medium modified by the IP NAAS - 478.0 and 322.7 mg versus 286.8 and 20.3 mg; 382.0 and 269.7 mg when grown on nutrient media MS and IIA NAAS, respectively. The yield of microtubers weighing more than 350 mg was 65.1; 57.0 and 47.7%, respectively (Fig. 1).

Mid-early ripening cultivar Levada showed significantly higher productivity on the nutritional medium modified by the IIA NAAS. So, the mass of the average microtube and the tubers mass per one plant was 431.5 and 343.7 mg, which is 1.8 and 3.5 times more than when cultivated on the MS nutrient medium and 3.7 and 4.7 times more than growing on IP NAAS.

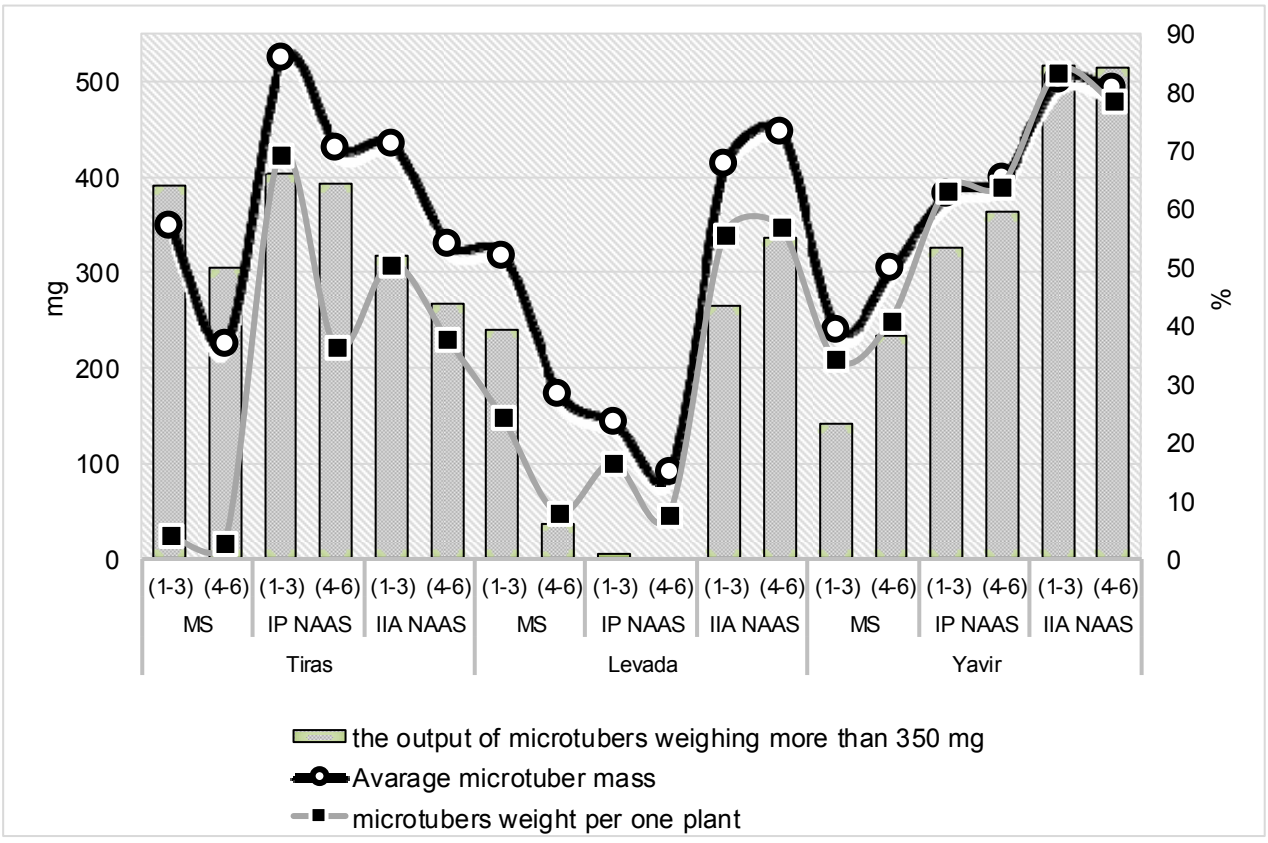

Fig. 1 productivity of cultivars of different ripeness groups depending of the nutrient medium and different parts of plants in in vitro conditions

In our research mid-ripening cultivar Yavir had the mass of the average microtuber was 497.5 mg versus 272.3 and 391.1 mg when grown on nutrient medium of MS and IP NAAS, respectively. The mass of microtubers per one plant one the nutrient medium modified by the IIA NAAS is 494.8 mg, which is 266.0 and 106.4 mg more than on MS and IP NAAN medium, respectively.

The weight of the average microtuber and the mass of microtubers per one plant differ when using cuttings of different tiers, so on the plants of cuttings of tires 1-3 the mass of the middle microtuber is 267.6 mg, which is 46.1 mg higher then at tier 4-6; the mass of microtubers per one plant is 272.4 mg (tier 1-3) against 225.3 mg (tier 4-6).

Conclusions.

So, according to the results of two years of research, the best productivity showing were obtained by cultivating the microtubers of the medium-ripened potato cultivar Yavir on the nutrient medium of the IIA NAAS from 1-3 tiers of steems in *in vitro* culture. In this case, the mass of the average microtuber was 502.0 mg; microtubers weight per plant - 509.8 mg.; the output of microtubers weighing more than 350 mg is 84.7%; the intensity of tuber formation was 101.5%.

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