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Improvement of the potato seed production system on a virus-free basis in Almaty region

This article presents data on the induction of potato microtubers and the potency of different varieties to form microtubers *in vitro*. It has been established that the main factors influencing the process of tuberization in potato regenerated plants are: the concentration of carbohydrates and plant growth regulators in the nutrient medium and physiological state. Experiments were carried out to obtain microtubers from regenerated plants of 4 potato varieties of domestic selection (Alliance, Babaev, Miras and Pamyaty Konaeva) for the selection of virus-free primary material for seed production.As a result, a modified nutrient medium Murasige and Skoog (MS) containing 20 mg/L orotic acid was developed to accelerate the formation of microtuber formation of potato plants *in vitro*.

Keywords: Solanum tuberosum L., microtubers, nutrient medium, *in vitro*.

Introduction

In Kazakhstan, potato (*Solanum tuberosum* L.) is a main food product, and take the second place in terms of importance after bread. Potato planting areas in the republic are approximately 195.0–200.0 thousand ha, the total annual harvest is 3.8–4.0 million. tons, and productivity — 18-19 t/ha. If the consumption rate per person is 100 kg, then it is known that we will meet the needs of the population of the republic in marketable potatoes. However, today providing the country's farms with high-quality seed material of potato crops cured of various diseases is becoming an urgent and insoluble problem. One of the reasons for the unsatisfactory situation in potato production is the lack of quality seed material. In many countries, including the Republic of Kazakhstan, during the last 40 years, virus-free healing technology has been introduced into the cultivation of potato seeds using the tip tissue method [1]. *In vitro* production of microtubers has a great future in order to create a stock and collection of potato varieties cured from the mentioned diseases, to preserve them and to increase efficiency in seed production [2].

In vitro method of obtaining microtubers is the most effective way for rapid multiplication of potatoes. According to many authors, the use of microtubers as a planting material in the field will undoubtedly greatly simplify the process of seed production [3, 4]. Compared to standard potato seed tubers, microtubers are superior. First of all, the microtuber removal method prevents its re-infection, so they are completely neutralized from the pathogen. Due to their small size and weight, they are very convenient for storage and transportation. Microtubules can be obtained in artificial culture medium at any time in the laboratory. Storage of a collection of varieties in the form of microtubers ensures their complete isolation from pathogens [4]. Many researchers have shown the importance of nutrient medium composition for the induction of healthy microtubules *in vitro* [5].

According to scientists' research, the use of substances that regulate growth activity in modern technologies of potato cultivation is an important factor that increases its reproduction rate and productivity [6]. Growth regulators for the treatment of vegetative plants are a type of biological catalyst, immunomodulator and adaptogen [7]. They activate vital physiological processes of plants, increase their productivity, and also provide resistance to diseases and adverse environmental stresses [8]. The main regulatory factor of plant growth is their growing nutrient medium, and an important condition for plant growth is the presence of plant growth regulators (PGR) in the nutrient medium [9].

In this situation, it is very important to find effective ways to optimize the process of breeding unique seeds treated for diseases, to find and effectively use the most productive and economical ways to obtain treated raw materials in order to reduce the required material, labor and energy resources and reduce the cost of production.

The purpose of the research is to obtain microtubers from regenerated plants of domestic potato varieties cured of diseases *in vitro* and optimize the composition of the nutrient media for the rapid production of microtubers from regenerated plants on the basis of microclonal propagation.

Experimental

In cooperation with scientists from the department of potato breeding, seed production and biotechnology of the regional branch of the KazNII of the fruit and vegetable industry, "Kainar" LLP, research work was carried out in the direction of improving the virus-free potato seed production system.

There are 1,800 samples from 35 countries of the world in the potato gene pool of the "Kaynar" regional branch, including samples of 46 wild and 279 cultivated types of potatoes. According to the origin, half of it or 50% of varieties and interspecies hybrids of Russian selection, 19% of European countries, 18% of Kazakh selection, 13% of the rest of the world. Therefore, the development of seed production of promising domestic potato varieties adapted to the soil and climatic conditions of our country is an urgent issue. As an object of study, we used regenerated plants grown in vitro from potato varieties of domestic selection Alliance, Babaev, Miras and Pamyaty Konaeva, which are characterized by high productivity, disease resistance and are more adapted to the soil and climatic conditions of the Almaty region, as well as selected for results of market research and demand from farmers.

The method of isolation of apical meristems was used to obtain the initial material of potatoes according to the recommendations of domestic and foreign scientists [2–5]. Murasige and Skoog (MS) medium was modified and purified according to the components. MS medium supplemented with high concentrations of sucrose and different concentration and combination of PGR (6-benzylaminopurine (BAP), kinetin, adenine, heteroauxin) used as inducers of microtuber formation. Increase the concentration of carbohydrate in MS medium was achieved by using the following concentrations of sucrose 40.000 mg/L, 60.000 mg/L, 80.000 mg/L, 100.000 mg/L, 120.000 mg/L and 140.000 mg/L or from 4 up to 14%. MS medium containing 20,000 mg/L (20%) sucrose was used as a control. In the course of research, the influence of sucrose and PGR concentration in the nutrient medium on the formation of tubers, as well as the temperature regime during storage of microtubers, was determined. In order to obtain potato microtubers *in vitro*, the scientists of the regional branch of the KazNII of the fruit and vegetable industry, "Kainar" LLP, increased the content of sugars, changed the content of phytohormones in the nutrient medium, etc. according to the developed recommendations and methods of microtuber induction [9, 10].

The evaluation of the results was determined when tubers appeared on the roots of the plant. The regenerated plants were grown under aseptic conditions, in laminar boxes, in glass test tubes and flasks filled with a nutrient medium with tuberization inductors. The influence of various physical factors on the growth of plants and the formation of microtubules in them is considered. The grafted plants were initially placed in a permanently dark chamber, and after 4-5 days they were transferred to the light, the photoperiod was 16/8 hours. Plants were grown in a phytotron at a temperature of 22°C.

At the initial stage of microsprout cloning, the 1st cultivation was carried out in September using initial plants isolated from the in vitro collection. The 2nd and 3rd cultivation was carried out in October and November. All parts of the plants, upper, middle and lower, were used to obtain microtubers.

Results

Studies have made it possible to determine the potency of various potato varieties to form tubers in vitro. The ability to form microtubers depended primarily on the biological characteristics of varieties, growing conditions, the optimal combination of PGR, the concentration of sucrose and other components that positively contribute to the formation of tubers.

The results of the study showed that with an increase in the content of sucrose in the nutrient medium to 80.000 mg/L, tuberization in all tested potato varieties reached about 60.0% (Fig. 1).

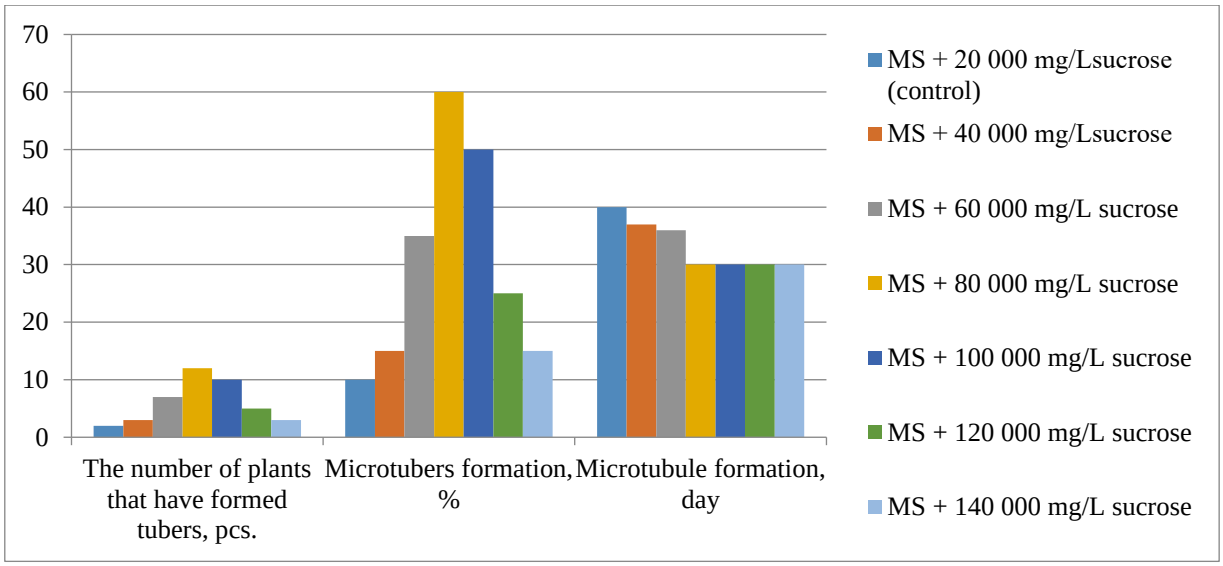

Figure 1. Foration of microtubers in potato plants on MS-medium with different concentrations of sucrose.

It has been established that an increase in the concentration of sucrose (100.000, 120.000 and 140.000 mg/L) in the nutrient medium leads to a decrease in tuberization of potato plants. Thus, it was found that the amount of carbohydrates in the artificial nutrient medium, exceeding 80.000 mg/L, adversely affects the formation of microtubers in potato plants. In addition, it was found that the time of formation of microtubers in the studied varieties in all variants was 30–40 days, and the concentration of carbohydrates in the nutrient medium did not contribute to the acceleration of tuberization.

At the next stage of the study, the effect of various concentrations of sucrose on the formation of the biomass of microtubules formed *in vitro* was determined. As a result of the study, it was found that the addition of 8% or 80.000 mg/L of sucrose to the MS medium showed a positive effect on increasing the number, size and mass of microtubers in plants.

The data in Table 1 show that the biological characteristics of potato varieties have a significant impact on the ability of plants to form microtubers. The number of microtubers of varieties Alliance, Babaev, Miras and Pamyaty Konaeva was 1.6–1.9 pcs per plant in the variant of MS medium with 80,000 mg/l of sucrose, and in the control variant only 0.8–1.0 pieces of microtubers were formed per plant.

Table 1

Effect of different concentrations of sucrose in the MS medium on biomass of microtubers obtained *in vitro* from potato varieties

Variety	Nutrient media	The number of microtubers per 1 plant, pcs.	Size of 1 microtuber, mm	Weight of 1 microtuber, mg
Alliance	MS + 20 000 mg/Lsucrose (control)	1.0	5.0	163.0
	MS + 40 000 mg/Lsucrose	1.0	5.3	168.2
	MS + 60 000 mg/L sucrose	1.3	5.5	189.0
	MS + 80 000 mg/L sucrose	1.7	6.0	196.7
Babaev	MS + 20 000 mg/L sucrose (control)	0.8	5.1	159.0
	MS + 40 000 mg/L sucrose	0.9	5.6	182.3
	MS + 60 000 mg/L sucrose	1.3	5.8	186.7
	MS + 80 000 mg/L sucrose	1.9	6.1	193.1
Miras	MS + 20 000 mg/L sucrose (control)	1.0	5.2	164.0
	MS + 40 000 mg/L sucrose	1.0	5.3	166.5
	MS + 60 000 mg/L sucrose	1.2	5.5	174.2
	MS + 80 000 mg/L sucrose	1.6	6.5	188.7
Pamyaty Konaeva	MS + 20 000 mg/L sucrose (control)	1.0	5.0	160.0
	MS + 40 000 mg/L sucrose	1.3	5.3	166.0
	MS + 60 000 mg/L sucrose	1.4	5.8	179.3
	MS + 80 000 mg/L sucrose	1.9	6.2	200.0

Depending on the genotype of the tested potato varieties, the volume of 1 microtuber in the experimental variants was 5.3–6.2 mm, and in the control ― 5.0–5.1 mm. The weight of 1 microtuber in the experimental variant was 166.0–200.0 mg, in the control ― 159.0–164.0 mg.

The highest indicator in terms of the number of microtubers per 1 plant, the size and weight of microtubers was observed on the variant of the MS medium with 80,000 mg/l of sucrose. Among the tested cultivars, the cultivar Pamyaty Konaeva according to the specified version stood out with high rates. The number of tubers per 1 plant of the named variety in the experimental variant was 1.9 pcs, in the control variant ― 1.0 pcs. The volume of 1 microtuber was 6.2 mm in the experimental variant and 5.0 in the control variant, and the weight of 1 tuber was 200.0 mg in the experimental variant and 160.0 mg in the control.

To accelerate the formation of microtubers, various concentrations of orotic acid (from 10 to 30 mg/L) were added to the artificial nutrient medium, and an effective variant of the medium was identified. Murasige-Skoog medium supplemented with 80,000 mg/L sucrose was used as a control. It was established that the addition of 20 mg/L of saturated orotic acid to the nutrient medium contributed to the formation of plant microtubers in vitro in a short period of time. In addition, it was found that the degree of tuberization in the selected experimental variant was higher than in the control (Table 2).

Table 2

The effect of orotic acid on *in vitro* tuberization in potato varieties

Nutrient media	Microtuber formation, %			
	10 days	20 days	30 days	40 days
Alliance				
MS + 80 000 mg/L sucrose (control)	23.5	32.0	50.0	56.0
MS + 80 000 mg/L sucrose+ 10 mg/Lorotic acid	32.0	35.0	52.0	64.6
MS + 80 000 mg/L sucrose + 20 mg/L orotic acid	38.0	49.9	63.0	79.0
MS + 80 000 mg/L sucrose + 30 mg/L orotic acid	38.0	50.0	63.0	79.0
Babaev				
MS + 80 000 mg/L sucrose (control)	29.0	33.0	48.0	52.0
MS + 80 000 mg/L sucrose + 10 mg/L orotic acid	32.3	33.6	49.8	57.0
MS + 80 000 mg/L sucrose + 20 mg/L orotic acid	35.0	51.0	76.8	80.0
MS + 80 000 mg/L sucrose + 30 mg/L orotic acid	35.0	51.0	76.0	78.0
Miras				
MS + 80 000 mg/L sucrose (control)	28.9	34.0	44.5	49.0
MS + 80 000 mg/L sucrose + 10 mg/L orotic acid	36.0	58.0	66.0	86.2
MS + 80 000 mg/L sucrose + 20 mg/L orotic acid	44.0	89.8	100	-
MS + 80 000 mg/L sucrose + 30 mg/L orotic acid	44.1	89.8	980	-
Pamyaty Konaeva				
MS + 80 000 mg/L sucrose (control)	30.0	30.0	48.0	53.2
MS + 80 000 mg/L sucrose + 10 mg/L orotic acid	37.2	56.8	68.1	85.3
MS + 80 000 mg/L sucrose + 20 mg/L orotic acid	45.2	83.6	100	-
MS + 80 000 mg/L sucrose + 30 mg/L orotic acid	45.2	83.6	100	-

According to the results of the study, it was found that a nutrient medium containing orotic acid at a concentration of 20.0 mg/l is optimal for the rapid formation of microtubers in all potato varieties. In this variant, the formation of microtubers of potato varieties Miras and Pamyaty Konaeva for 30 days was 100%, and in the control variant, the formation of microtubers of these varieties on the 40th day was only 49.0–53.2%. In the experimental variant, the formation of tubers in potato plants of the Alliance variety was 79.0%, and in the Babaev variety — 80.0% in 40 days.

Thus, in the process of accelerating the process of microtuber formation, the effectiveness of the MS nutrient medium containing 20 mg/L of orotic acid was shown. This modified Murasige and Skoog nutrient medium has been proposed to accelerate the formation of microtubers in potato plants *in vitro* (Table 3).

Table 3

The composition of the modified MS medium to accelerate the growth of microtubers of potato varieties *in vitro*

№	Components	Concentration, mg/L
Macroelements		
1	NH_4NO_3	1650
2	KNO_3	1900
3	$CaCl_2 . 2H_2O$	440
4	$MgSO_4 . 7H_2O$	-
5	KH_2PO_4	370
6	Na_2ЭДТА	170
7	$FeSO_4 * 7H_2O$	37,3
Microelements		
8	H_3BO_3	6,2
9	$MnSO_4 . 4H_2O$	22,3
10	$KnSO_4 . 4H_2O$	8,6
11	KI	0,75
12	$CuSO_4 . 5H_2O$	0,025
13	$Na_2MoO_4 . 2H_2O$	0,25
14	$CoCl_2 . 6H_2O$	0,025
Vitamins		
15	Thiamine (B_1)	1,0
16	Ascorbic acid (C)	2,0
17	Pyridoxine (B_6)	1.0
Growth regulators		
18	Orotic acid	20,0
19	Gibberellic acid	2 mg/L
Carbohydrates		
20	Sucrose	80 000or 8%
Other reagents		
21	Casein hydrolysate	40,0 g
22	Agar-agar	6-7 g
	pH	5,7-5,8

The data of many studies show that potato microtubers appear only in cuttings taken from the middle and lower parts of plants [6–8]. In our studies, it was found that most microtubers are formed in the middle part of potato plants.

Discussion

Experiments were carried out on the induction of microtubers from potato regenerated plants obtained *in vitro* from varieties of domestic selection Alliance, Babaev, Miras and Pamyaty Konaeva. Two types of tuberization have been identified: the first one is in plant shoots developing from cuttings; the second — in the axils of the leaves of plants grown from cuttings.

During the experiment, we observed that growing cuttings at a 16-hour light period, and then transferring them to a dark place, then micronodules usually appear on the shoots. If, after planting, the cuttings of the plant were immediately transferred to a dark place, microtubers develop mainly in the axils of the leaves [10].

Conclusion

In the process of optimizing the conditions for obtaining microtubers from plants grown on an artificial nutrient medium in vitro, it was shown that tuberization in all tested potato varieties was 60.0% with an increase in the sucrose content in the nutrient medium to 80.000 mg/L. As sucrose saturation increased (100.000, 120.000 and 140.000 mg/L), a decrease in the ability of potato plants to form tubers in a nutrient medium was observed. Thus, the concentration of carbohydrates in the nutrient medium in excess of 80,000 mg/L has a negative effect on the formation of microtubers in potato plants.

It was shown that when studying the formation of microtubers in potato varieties in vitro, an increase in sucrose to 80,000 mg/L positively affects the increase in the number, size and weight of microtubers formed on plants. The addition of 20.0 mg/L of saturated orotic acid to the culture medium as an inducer contributed to the acceleration of microtubule formation, showing higher rates compared to the control. In this variant of the medium, depending on the biological characteristics of the variety, 100% of microtubers were formed after 20, 30 days, and in the control variant, the formation of microtubers reached 95% only on the 40th day. As a result of the study, a modified Murasige and Skoog nutrient medium was proposed for the rapid induction of potato microtubers in vitro.

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Алматы облысы жағдайында картоптың тұқым шаруашылығының вируссыз негіздегі жүйесін жетілдіру

Мақалада *in vitro* жағдайда картоп микротүйнектерін индукциялау және әртүрлі сорттардың микротүйнек түзу қабілеттері туралы деректер берілген. Картоптың өсімдіктер-регенеранттарынан түйнектердің пайда болуына әсер ететін негізгі факторлар қоректік ортадағы көмірсулар мен өсімдіктердің өсу реттегіштерінің концентрациясы және өсімдіктің физиологиялық жағдайы екені анықталды. Отандық селекциядағы картоптың *Альянс, Бабаев, Мирас* және *Памяти Конаева*, яғни осы 4-сорттың микротүйіндерін алу үшін тәжірибелер жүргізілді. Нәтижесінде *in vitro* картоп өсімдіктерінде микротүйінділердің түзілуін жеделдету үшін құрамында 20 мг/л орот қышқылы бар модификацияланған *Мурасиге* және *Скугаға* қоректік орта жасалды.

Кілт сөздер: *Solanum tuberosum* L., микротүйнектер, қоректік орта, *in vitro*.

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Совершенствование системы семеноводства картофеля на безвирусной основе в условиях Алматинской области

В статье приведены данные об индукции микроклубней картофеля в культуре *in vitro,* и выявлена способность различных сортов формировать микроклубни. Установлено, что основными факторами, влияющими на клубнеобразование в растениях-регенерантах картофеля, являются концентрация углеводов и регуляторов роста растений в питательной среде и физиологическое состояние растения-регенеранта. Проведены опыты по получению микроклубней 4-х сортов картофеля отечественной селекции: *Альянс, Бабаев, Мирас* и *Памяти Конаева.* В результате разработана модифицированная питательная среда *Мурасиге* и *Скуга*, содержащая 20 мг/л оротической кислоты, для ускорения образования микроклубней у растений картофеля *in vitro*.

Ключевые слова: Solanum tuberosum L., микроклубни картофеля, питательная среда, *in vitro,*отечественная селекция.

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