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D. Volkov, A. Argynbayeva, D. Daurov, K. Zhapar, Zh. Abai, K. Zhambakin, M. Shamekova

Institute of plant biology and biotechnology, Almaty, Kazakhstan.
E-mail: m.shamekova@gmail.com, volkovdmitriykz@gmail.com, asselargynbayeva@gmail.com, dias.daurov@gmail.ru, zhapar.zk@gmail.com, abaizh097@mail.ru, zhambakin@gmail.ru, m.shamekova@gmail.com

ACCELERATED PRODUCTION OF VIRUS-FREE POTATO PLANTING MATERIAL USING A BIOREACTOR

Abstract. Potato production is one of the key branches of crop production that determines the food security of Kazakhstan. The Republic needs over 800,000 tons of seed potatoes per year. In addition to seed potatoes, which are grown in Kazakhstan, about 30,000 tons of seed potatoes are imported annually, while about 80% of this volume is imported from the Netherlands through private companies [1].

In 2018, 193.0 thousand hectares were occupied under potatoes in Kazakhstan, while the gross harvest amounted to 3806.9 thousand tons. At the same time, the yield in 2018 was only 19.8 t/ha. While in neighboring Uzbekistan in 2018, the yield was 33.68 t/ha, the maximum yield in New Zealand in 2018 was about 50.41 t/ha[2]. It is known that one of the main reasons for low potato yield is low-quality seed material.

In Kazakhstan, mainly after obtaining virus-free plants *in vitro* through meristem culture, minitubers are obtained from them in most technological processes; in rare cases, microtubers are obtained from meristem plants *in vitro* and then minitubers from them.

Research has shown that the bioreactor can massively clone meristem plants and get full-fledged virus-free microtubules reducing a significant proportion of manual labor, thereby reducing the impact on the result of the human factor, reduce infections, and reduce labor costs and material costs.

Key words: potato, microtubers, minitubers, virus-free culture, DAS-ELISA.

Introduction. The main requirement for quality seed material is the absence of pathogenic and quarantine diseases. There are about 40 types of viruses and 2 viroids that affect potatoes [3]. Depending on the defeat of viral diseases, the yield drops to 90% on production crops [4].

Healthy and high-quality potato seeds are the basis of potato seed production [5]. First of all the seed material must be free of pathogenic microorganisms.

After obtaining virus-free plants *in vitro* through meristem culture, in most technological processes, minitubers are obtained from them. The production of mini-tubers is the final stage of obtaining virus-free material [6].

Recently, the production of microtubers is often used from which, as from meristem plants *in vitro*, minitubers are obtained.

Microtubers are the result of *in vitro* cultivation of plants in an artificial nutrient medium [7]. Many studies are aimed at improving the efficiency of obtaining microtubers and increasing their size, for example, by cyclically immersing plants in a liquid nutrient medium during tuber formation [8]. At the same time, despite a sufficient number of publications on the production of microtubers *in vitro*, there is still little information on their testing in the ground [9]. In global seed production, minitubers are currently an intermediate between the production of meristem plants and microtubers *in vitro* and field propagation of seed material. The production of seed potatoes using minitubers requires much stricter control of the resistance of the planting material to abiotic and biotic stress factors [10]. When planting mini-tubers

directly in the field, their size is of great importance [11] and Rykaczewska [12] found that the larger the minitubers, the more uniform the seedlings, the higher the yield and the dry mass content.

Methods. Isolating the apical meristem. Excised shoot tips collected from actively growing twigs wash under running tap water and disinfect with 0.1% mercuric chloride solution containing approximately 0.02% Tween-20 for 6 min inside a running laminar air flow cabinet. Treated explants wash four to five times with sterile distilled water to remove the effect of the sterilizing agent. Shoot apical meristem consisting of the apical dome with one to two leaf primorida isolates using sterile hypodermic needle and scalpel under a dissecting microscope. To avoid dehydration isolated meristems (0.3–0.5 mm) transfer quickly on the filter paper bridge in test tubes containing sterilized liquid MS medium with the addition of kinetin 2 mg / l and 0.5 mg/ l gibberellic acid. After 4 weeks, the developed meristems subculture on semisolid medium with the addition of kinetin 3 mg/l and gibberellic acid 0.5 mg/l for further growth for shoot elongation and root formation [13,14,15]. After 2-3 weeks received plantlets transplanted into semisolid MS medium without hormones supplemented with vitamins, 3% sucrose, 0.8% agar, pH 5,7. After 4 weeks of culture on MS medium without hormones plantlets were cloned for further propagation and testing.

The cultivation in bioreactor

A hundred single explants are transferred to a bioreactor with 1000 ml of liquid medium with 30 g/l of sucrose and cultivated for 4 weeks with constant illumination about 2.5 W/m^2. Explants are grown to 15 cm. Then the medium is changed to 8000 ml of a liquid medium with 90 g/l of sucrose and cultivated for 6 weeks with constant illumination about 0,9 W/m^2 at 25 °C. The medium enters the bioreactor every 6 hours and is present for 1 hour, so explants absorb the liquid medium only 1 hour every 6 hours. The bioreactor is aerated with sterile air from the calculation of 1 ml/min of air per 10 ml of liquid medium [16].

Total DNA extraction

Extraction of DNA from the plants is performed using the manufacturer's instructions commercial for nucleic acid extraction kits or CTAB method [17].

Total RNA extraction

Extraction of RNA from the plants is performed using the manufacturer's instructions commercial for nucleic acid extraction kits [18].

Reverse transcription reaction isolated RNA

The reaction of reverse transcription extracted RNA is performed using the instructions attached to Sileks reagents [19].

Double Antibody Sandwich ELISA (DAS-ELISA) will be done using commercial kits according to the manufacturer's instructions [20].

Results and discussions. After isolation of the apical meristem of potatoes during 30 days of cultivation, meristem plants of five varieties (Minerva, Romano, Aladin, Soprano from the Netherlands) and (Nevsky from Russia) were obtained, which were checked for the absence of PVM, PVS, PVX, PVY viruses by PCR and ELISA analysis (table 1).

Thus, plants that were pure for all four viruses were selected, which were cloned *in vitro* and used to produce microtubers in a bioreactor. Healthy plants were divided into nodal segments and placed in a bioreactor (10 nodal segments of each variety in three repetitions) with a liquid nutrient medium optimized by MS with sucrose 30 g/l, kinetin 2 mg/l and gibberilinic acid 0.5 mg/l where they were cultivated for 30 days at a temperature of 25°C, light mode 16/8 day/night.

Then the plants obtained from the nodal segments were cultivated in a bioreactor with a liquid nutrient medium MS with sucrose 90 g/l and kinetin 2 mg/l at 18°C, light mode 0/24 day/night for 60 days before harvesting microtubers.

The formation of microtubers in different varieties began in about 15-20 days, the harvest was collected on day 60.

Table 1 – Testing of meristem plants for the presence of viruses for further cultivation in a bioreactor.

Variety	Virus	RT-PCR Multiplex		IFA	
		Quantity of positive samples, PCs	% relation	Quantity of positive samples, PCs	% relation
Minerva	PVM	0	0	0	0
	PVS	0	0	0	0
	PVX	0	0	0	0
	PVY	2	25	1	12.5
Romano	PVM	0	0	0	0
	PVS	0	0	0	0
	PVX	0	0	0	0
	PVY	0	0	0	0
Aladin	PVM	7	28	7	28
	PVS	3	12	0	0
	PVX	1	4	1	4
	PVY	3	12	2	8
	PVM/PVS	1	4	0	0
	PVM/PVY	1	4	1	4
	PVM/PVS/PVY	1	4	0	0
	PVM/PVX/PVY	1	4	1	4
Soprano	PVM	19	61.29	15	48.38
	PVS	10	32.2	2	6.45
	PVX	0	0	0	0
	PVY	0	0	0	0
	PVM/PVS	10	32.2	2	6.45

Table 2 – The formation of potato microtubers in the bioreactor

Variety	The beginning of the formation of microtubers	Quantity (PCs/plant)	Weight of microtuber (g)
Minerva	18	0,7(±0,48)	0,169(±0,017)
Romano	19	0,8(±0,63)	0,143(±0,014)
Aladin	21	0,5(± 0,53)	0,65(±0,007)
Soprano	15	1(±0,67)	0,310 (±0,021)
Nevsky	17	1,2(±0,63)	0,156(±0,008)

Depending on the genotype, the difference in the beginning of microtuber formation in the bioreactor after placing plants in the dark phase was 6 days, the largest microtubers were in the Aladin variety – 0.65 (±0.007) g, then in Minerva 0.310 (±0.021) g and less than 0.2 g in Romano, Aladin and Nevsky.

Microtubers obtained in the bioreactor were analyzed for the presence of PVM, PVS, PVX, and PVY. As a result, 2 samples of the Aladdin variety infected with PVM were detected in one of three replications (table 3). The microtubers were selected one from each of the plants.

Table 3 - Checking microtubers obtained in the bioreactor for the presence of viruses

№ of samples	Viruses							
	PVM		PVS		PVX		PVY	
	PCR	IFA	PCR	IFA	PCR	IFA	PCR	IFA
1	2	3	4	5	6	7	8	9
Minerva								
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-

table continuation 3

1	2	3	4	5	6	7	8	9
Aladin								
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
5	+	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-
9	+	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-
Romano								
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-
Soprano								
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-
Nevsky								
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-

Analysis for the presence of viruses in microtubers showed that control is necessary at this stage, since microtubers are piece material and getting infected material into the further process will allow mass replication of viruses in the seed material.

The virus-free microtubers obtained in the bioreactor were stored and stratified for 6 months in dark conditions at a temperature of 4°C. Then the microtubers were placed in the light at a temperature of 20 to 25°C for 30 days until the shoots appeared and transplanted into pots in controlled conditions of the greenhouse for 15 days until the plants reached the phase 5 leaves and then transplanted into the open ground for 30 pieces of each variety. Harvesting of minitubers was carried out 3 months after planting seedlings in the open ground.

According to the results of morphological analysis of minitubers (figure 1, table 4), they was smooth without flaws and standard for further seed production and the maximum number of them was in Soprano and Nevsky varieties, the average in Minerva and Romano, and the minimum in Aladin.

Table 4 – Morphological parameters of minitubers

№	Name of the variety	Quantity of minitubers from plants, PCs	Weight of the tuber, g
1	Soprano	8,1(±2,4)	24(±16,3)
2	Nevsky	9(±3,9)	12,9(±5,6)
3	Aladin	3,6(±1,4)	5,84(±4,9)
4	Minerva	5,5(±1,9)	13,1(±3,4)
5	Romano	6(±2,9)	11,2(±10)

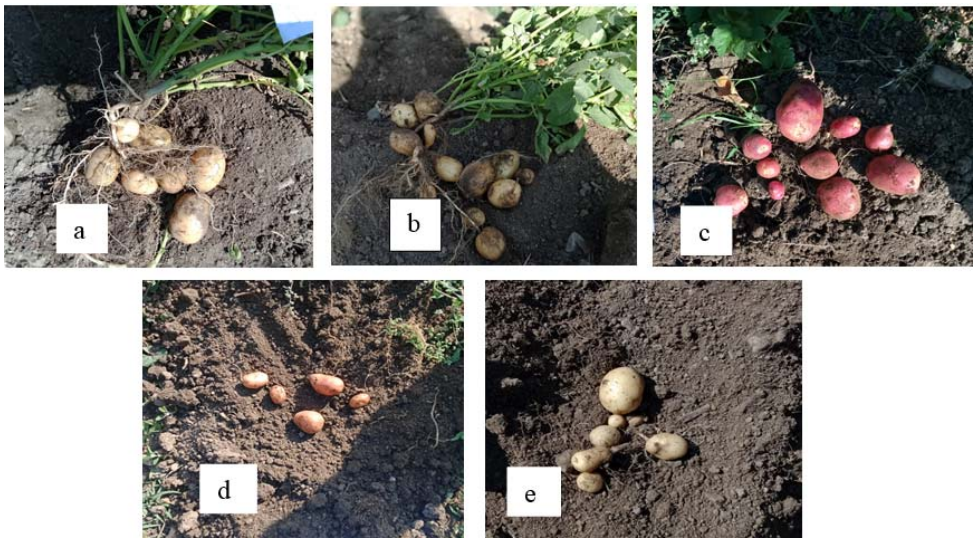

Figure 2 – Minitubers of varieties: a - Soprano, b - Nevsky, c - Aladin, d - Minerva, e - Romano

From the conducted research, it can be concluded that with the help of a bioreactor, it is possible to obtain high-quality microtubers from which high-quality virus-free minitubers will be obtained. The process can be accelerated by earlier collection of microtubers from the bioreactor, for example, after 45 days, since all 5 varieties had normally formed microtubers at 45 days. In addition, studies have shown that the bioreactor can massively clone meristem plants and get full-fledged virus-free microtubers, reducing a significant proportion of manual labor, thereby reducing the impact on the result of the human factor, reducing infections, reducing labor costs and material costs.

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Д.В. Волков, Ә.М. Арғынбаева, Д. Л. Дауров,
Қ.Қ. Жапар, Ж.С. Абай, Қ.Ж. Жамбакин, М.Х. Шамекова

Өсімдіктер биологиясы және биотехнологиясы институты, Алматы, Қазақстан

БИОРЕАКТОРДЫҢ КӨМЕГІМЕН КАРТОПТЫҢ ВИРУССЫЗ ОТЫРҒЫЗУ МАТЕРИАЛЫН ЖЕДЕЛДЕТІП ӨНДІРУ

Аннотация. Картоп өсіру шаруашылығы – Қазақстандағы азық-түлік қауіпсіздігін анықтайтын өсімдік шаруашылығының негізгі салаларының бірі. Республикаға жылына 800000 тоннаға дейін тұқымдық картоп қажет. Қазақстанда өсірілетін тұқымдық картоптан басқа, жыл сайын 30 мың тоннаға жуық тұқымдық картоп импортталады, оның 80% Нидерландыдан жеке компаниялар арқылы әкелінеді.

Қазақстанда 2018 жылы картоп 193,0 мың гектарды қамтыса да, жалпы өнім 3806,9 мың тонна болды. Сонымен бірге, 2018 жылы жалпы өнім 19,8 ц / га жетті. 2018 жылы көршілес Өзбекстанда өнімділік 33,68 т/га

болса, Жаңа Зеландияда жоғары өнімділік 2018 жылы шамамен 50,41 т/га құраған. Картоп өнімінің азаюының басты себебіне сапасыз тұқым материалы жататыны белгілі. Соңғы кезде *in vitro* меристемалы өсімдігінен шағын түйнектер алынды, соның ішінде микро-түйнек өндірісі қолданылады. Микротүйнектер жасанды қоректік ортада invitro өсімдіктерін өсіргенде пайда болады.

Шағын түйнек – *in vitro* меристемалы өсімдіктен немесе микротүйнектен алынатын кішкентай түйнек. Отырғызғанда әртүрлілігі мен тығыздығына байланысты мөлшері 10-нан 50 мм-ге дейін өзгереді. Шағын түйнектің тұқымдық құндылығы қоздырғыштардың болмағандығымен және мөлшері арқылы анықталады. Бір *in vitro* меристемалы өсімдіктен немесе жабық жердегі микротүйнектен 2-ден 10-ға дейін шағын түйнек, ал егер гидропоника қолданғанда -40-қа дейін шағын түйнек алуға болады. Әлемдік өндірісте шағын түйнек қазіргі уақытта меристемалы өсімдік, *in vitro* микротүйнегін алу мен тұқымдық материалдың дала әдісімен көбеюі арасындағы аралық байланыс болып саналады. Шағын түйнек арқылы тұқымдық картопты өндіру отырғызу материалының абиотикалық және биотикалық стресс факторына төзімдігін қатаң қадағалауды қажет етеді. Шағын түйнекті далаға тікелей отырғызғанда мөлшерінде ерекшелік пайда болады. Rykaczewska түйнегі неғұрлым көп болса, соғұрлым біркелкі көшет, кіріс пен құрғақ массаның мөлшерінің жоғары екені анықталды.

Қазақстанда, негізінен, меристемалық дақыл арқылы *in vitro* вируссыз өсімдіктер алғаннан кейін, көптеген технологиялық процестер арқылы минитүйнекше, сирек жағдайда меристемалық өсімдіктен микротүйнекше алынады, содан кейін одан минитүйнекше алуға мүмкіндік туады.

Зерттеулер көрсеткендей, биореактордағы меристемалық өсімдіктерді жаппай клондау жұмысы қол еңбегінің үлесін едәуір азайтады, осылайша адами фактордың зерттеу нәтижесіне әсерін, ластануды, еңбек және материалдық шығынды азайту арқылы толыққанды вируссыз микротүйнекше алуға болады.

Түйін сөздер: картоп, минитүйнек, вируссыз культура, ПЦР, DAS-ELISA.

Д.В. Волков, А.М. Аргынбаева, Д. Л. Дауров,
К.К. Жапар, Ж.С. Абай, К.Ж. Жамбакин, М.Х. Шамекова

Институт биологии и биотехнологии растений, Алматы, Казахстан

УСКОРЕННОЕ ПРОИЗВОДСТВО БЕЗВИРУСНОГО ПОСАДОЧНОГО МАТЕРИАЛА КАРТОФЕЛЯ С ПОМОЩЬЮ БИОРЕАКТОРА

Аннотация. Картофелеводство является одной из ключевых отраслей растениеводства, определяющих продовольственную безопасность Казахстана.Республике требуется до 800 000 тонн семенного картофеля в год. Помимо семенного картофеля, который выращивается в Казахстане, ежегодно импортируется около 30 000 тонн семенного картофеля, при этом около 80% из этого объема ввозится из Нидерландов через частные компании.

Под картофелем в Казахстане 2018 году было занято 192,3 тыс. га при этом, валовый сбор составил 3806,9 тыс. тонн. При этом урожайность в 2018 году составила только 19,8 т/га. В то время как в соседнем Узбекистане в 2018 году урожайность составила 33,68 т/га, максимальная урожайность в Новой Зеландии в 2018 году была около 50,41 т/га. Известно, что одной из основных причин низкой урожайности картофеля является некачественный семенной материал.

В последнее время часто используется производство микроклубней, из которых как из меристемных растений *in vitro* получают миниклубни. Микроклубни являются результатом культивирование растений *in vitro* в искусственной питательной среде.

Миниклубни представляют собой небольшие клубни, полученные из меристемных растений *in vitro* или из микроклубней. В зависимости от сорта и плотности посадки их размер колеблется от 10 до 50 мм. Семенная ценность миниклубней определяется отсутствием патогенов и размером. Из одного меристемного растения *in vitro* или микроклубня в закрытом грунте можно получить от 2 до 10 миниклубней, если использовать гидропонику - до 40 миниклубней. В мировом производстве миниклубни в настоящее время представляют собой промежуточное звено между получением меристемных растений и микроклубней *in vitro* и полевым размножением семенного материала. Производство семенного картофеля с помощью миниклубней требует гораздо более строгого контроля устойчивости посадочного материала к абиотическим и биотическим стрессовым факторам. При высадке миниклубней непосредственно в полевые условия, большое значение имеет их размер. Rykaczewska обнаружила, что чем больше миниклубни, тем более равномерные всходы, выше урожай и содержание сухой массы.

Производство миниклубней является финальной стадией получения безвирусного семенного материала. В Казахстане в основном после получения безвирусных растений *in vitro* через культуру меристем в большинстве технологических процессах из них получают миниклубни, в редких случаях из меристемных растений получают микроклубни *in vitro* и затем из них миниклубни.

Исследования показали, что в биореакторе можно массово клонировать меристемные растения и получать полноценные безвирусные микроклубни сократив значительную долю ручного труда, тем самым снизить влияние на результат человеческого фактора, сократить заражения, сократить расходы на оплату труда и расходы на материалы.

Ключевые слова: картофель, миниклубни, безвирусная культура, ПЦР, DAS-ELISA.

Information about authors:

Dmitriy Vladimirovich Volkov: Institute of plant biology and biotechnology. Timiryazeva 45, Almaty, Kazakhstan. Senior researcher at the laboratory of breeding and biotechnology, master of biology; volkovdmitriykz@gmail.com; https://orcid.org/0000-0003-4609-7912;

Assel Mukhtarkyzy Argynbayeva: Institute of plant biology and biotechnology. Timiryazeva 45, Almaty, Kazakhstan. Junior researcher at the laboratory of breeding and biotechnology, master of biotechnology; asselargynbayeva@gmail.com; https://orcid.org/0000-0002-2436-6926;

Dias Lamzarovich Daurov: Institute of plant biology and biotechnology. Timiryazeva 45, Almaty, Kazakhstan. Research associate of the laboratory of breeding and biotechnology, master of biotechnology; dias.daurov@gmail.ru; https://orcid.org/0000-0003-3073-4577;

Kuanysh Kabyluly Zhapar: Institute of plant biology and biotechnology. Timiryazeva 45, Almaty, Kazakhstan. Junior researcher at the laboratory of breeding and biotechnology, master of biology; zhapar.zk@gmail.com; https://orcid.org/0000-0002-9007-9730;

Zhandos Sailaubekuly Abai: Institute of plant biology and biotechnology. Timiryazeva 45, Almaty, Kazakhstan. Laboratory assistant of the laboratory of breeding and biotechnology, bachelor of biotechnology; abaizh097@mail.ru; https://orcid.org/0000-0003-1822-1437;

Kabyl Zhaparovich Zhambakin: Institute of plant biology and biotechnology. Timiryazeva 45, Almaty, Kazakhstan.General Director of the RSE IPBB KN MES RK, academician of the NAS RK, doctor of biology, professor; zhambakin@gmail.ru; https://orcid.org/0000-0001-5243-145X;

Malika Khabidulayevna Shamekova: Institute of plant biology and biotechnology. Timiryazeva 45, Almaty, Kazakhstan.Head of the laboratory of breeding and biotechnology, associate Professor, PhD; m.shamekova@gmail.com; https://orcid.org/0000-0002-8746-7484

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