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Selecting sterilization conditions for the explants of different potato varieties to be *in vitro* introduced into the culture

An explant is a fragment of plant tissue or organ that is incubated on its own or used to produce a primary callus. The introduction of any plant's tissues into the culture *in vitro* begins with the selection of an uninfected viable explant. The authors presented the results of the effect of various sterilizing agents as well as the treatment timing on the viability of plant explants during microclonal propagation of potatoes of the following varieties: Aladin, Gala, Nevsky, Udacha and Kostanay Novosti. The meristem sprouts from potato tubers were used as parent material. The following commercial sterilizing agents were used: Bleach (Belizna), Tween 20, Lyzoformin 3000, 96 % ethyl alcohol. The influence of sterilizing agents as well as the timing of the treatment on the viability of explants and their contamination is analyzed. As a result of the experiments, it has been found that the most effective sterilizing agent is the 3 % bleach and 96 % ethyl alcohol with exposure duration of 10 minutes. Based on the results of the experiment, it is proposed to use the 3 % bleach with an exposure time of 10 minutes as the main sterilizing agent, and the 96 % ethyl alcohol as some pretreatment of explants to reduce surface contamination. The authors believe that the 96 % ethyl alcohol is the best option for sterilizing solutions since it is a non-toxic, gentle method of sterilization with minimum damage of the plant material tissues.

Keywords: potato tubers, meristem sprouts, sterilizing solutions, explant, exposure, sterilization.

Introduction

An important stage of microclonal propagation of plants and vegetable crops is the selection of the explant, its *in vitro* introduction into the culture and obtaining an aseptic culture. Epiphytic microflora and rhizosphere microorganisms [1] accompany the surface of cells, tissues and explants of plants and vegetable crops. When plant cells are introduced into callus culture, the problem of sterility of explants is acute. The selection of sterilization conditions is an important stage in all the works on the culture of isolated cells, tissues and explants of plants and vegetable crops. The choice of sterilizing substance, its concentration and exposure time are determined depending on the type of cells, tissue, explants of plants and vegetable crops.

In addition, the substance should not penetrate deeply into the tissue and should be easily washed out. When injected into culture *in vitro*, the sterilizing substance and exposure time are selected in such a way as to neutralize the concomitant epiphytic and rhizosphere microflora and at the same time not to significantly damage the explant tissues [2–4].

In the scientific literature [5–7], a wide range of different sterilizing substances is used: those containing active chlorine (calcium or sodium hypochlorite, chloramine), mercury preparations — sublimate, diacide, and oxidizing agents — hydrogen peroxide, potassium permanganate, etc.

Potatoes are one of the most important crops after wheat for the Republic of Kazakhstan [8]. Potato (*Solanum tuberosum*) is a species of perennial tuberous herbaceous plants from the *Solanum* genus of the *Solanaceae* family. The massive spread of bacterial, fungal, viral diseases on potatoes and the great damage caused by them to the yield and quality of tubers develop the need to produce seed materials by using active healing methods, such as microclonal propagation with a combination of cryopreservation of meristematic tissues in liquid nitrogen at the temperature –196 °C. These prerequisites will not only reliably preserve the potato gene pool for a long time, but also allow for obtaining the planting material during subsequent regeneration freed from phytopathogenic microorganisms [9–10].

Potatoes are heterozygous crops, which makes it difficult to preserve the genetic purity of a cultivated variety through continuous vegetative propagation [11].

To prevent the loss of potato genetic resources, the method of long-term storage of plant samples in gene banks, gene resource centers and cryo-collections is used in the CIS and foreign countries [12–14].

Storing and preserving genetic resources in gene banks is very important due to the high biological value as a breeding sample and for the further scientific research in biotechnology, crop production, and agriculture. Therefore, in order to preserve the genetic resource of the studied plant samples during long-term storage, the cryopreservation method is considered to be the best option today [15].

One of the advantages of cryopreservation storage at very low temperatures is the ability to significantly slow down or even stop metabolic processes and biological destruction in the cells of living organisms. In this case, the plant material remains genetically stable, which does not lead to genetic changes [16].

Currently, in the major gene banks worldwide, such as the *International Potato Center (CIP*), Argentina — INTA (INTA Balcarce Potato Collection); Czech Republic — CRI (Crop Research Institute) and PRI (Potato Research Institute, Havlíčkov Brod); Republic of Korea — NAC (National Agrobiodiversity Center); Peru — CIP (International Potato Center); Leibniz Institute of *Plant Genetics and Crop Research (IPK*), the Vavilov All-Russian Research Institute of Plant Production (VIR), the Scientific and Practical Center for Potato and Horticulture of the National Academy of Sciences of Belarus (SPC NASB) store potatoes using three storage systems: natural conditions (field collections), *in vitro*, at ultra-low temperatures (cryocollections) [17–18].

Thus, one of the stages of microclonal propagation that is the sterilization of plant explants is aimed at obtaining sterile explants and viable explants when introduced into culture *in vitro*, which is of great current interest.

The aim of the given study is to identify the effect of various types of sterilizing agents on the efficiency of disinfection of explants of different varieties in order to further obtain callus crops.

Experimental

The 5 varieties of potatoes (Fig.) of Kazakhstani selection, namely, Aladin, Gala, Nevsky, Udacha and Kostanay Novosti (*Kazakh Agrotechnical University named after S. Seifullin (Astana) (KazATU*) were selected to be the test subjects for the research.

Figure. Varieties of potatoes: Aladin, Gala, Nevsky, Udacha and Kostanay Novosti

Kostanay Novosti: The variety is medium-late, table purpose. The plant is tall, upright, medium foliage with the stems colored with anthocyanin. The nest is very compact. The color of the flowers is bright red-purple, which turns pale by the end of flowering. The berry formation is moderately abundant. The tubers are round-oval with small eyeholes, red peel, weakly mesh, and yellow flesh.

The potential yield is 45.0–50.0 tons/ha. The starch content is 14–20 %. The taste is good. It is relatively resistant to viral diseases, heat and drought. When tested, it is easily distinguishable from other varieties. Tubers are capable of long-term storage. It has been included in the State Register of Breeding Achievements of the Republic of Kazakhstan since 2007 and has been zoned in the Kostanay region since 2008 [19].

Gala: The Gala variety is classified as early-ripening. It takes 75 to 80 days for it to ripen after planting. The plants have a spherical shape, blooming with white flowers. The stems are dense, dark green. Medium-

sized potato tubers grow up to 8 cm in length. Root vegetables are oval-shaped, yellow in the section, glossy dense peel with a waxy sheen.

Gala is a high-quality early-ripening table variety with beautiful round-oval and oval tuber shapes. The ripening period of the fully-ripen crop is only 70–80 days from the moment the seeds are planted in the ground. Tubers of medium size (average weight of one tuber is 100–120 grams), covered with a yellowish skin of medium thickness. The flesh is pale yellow to rich yellow, has a low starch content (11–13 %) and good taste. Gala is a high-yielding variety (up to 25 tubers from one plant) and highly marketable (98 %) with numerous advantages. The yield of marketable tubers on the 40th day after germination is 170 tons/ha. At the end of the growing season the total yield reaches 700 t/ha.

Aladin is a high-yielding mid-season table potato variety with round-oval tubers. The ripening period is 100–110 days. Aladin belongs to the Dutch selection. Potato tubers are large. The color of the peel is red; the flesh on the cut has a light cream color. Aladin is very unpretentious to growing conditions and is suitable for planting in loamy and sandy soils. Aladin is highly resistant to late blight, resistant to nematode. The starch content is 21 %. It has good palatability traits. Tubers have shallow eyeholes, marketable condition and are well stored. The yield of Aladin at the end of the growing season reaches 780 quintals per hectare.

Nevsky. It is a medium-early table variety. The period from germination to the technical ripeness of the tubers is 70–80 days. Tubers have an oblong-rounded shape with even peel without any roughness, white-yellow in color with slightly pinkish unburied eyeholes. On the cut of the tuber, a delicate white flesh is visible, which does not darken for a long time. The average weight of the tuber is 90–130 g. It is precisely because of the attractiveness of the tubers that this potato has earned recognition. Besides, it is easy to clean, wash and it does not fall apart when cooked. The potato has good storability (better than that of many mid-late and late varieties). No special storage conditions are required; the tubers do not sprout for a long time and retain their commercial appearance throughout the winter. Potato plants are lush, of medium height, semi-upright, of intermediate type. The stems are well-leafed with medium-sized light green leaves with a slight wavy edge. The inflorescences are compact, consisting of many small white flowers. The flowering of the plants is very abundant but short-lived. It has been zoned in the East Kazakhstan region since 1987.

Udacha is an early variety that reaches maturity in 55–60 days after planting. It is characterized by high yield — 42 tons/hectare. The plant has a strong stem and dense leaves. It blooms for a short time, with white inflorescences and bent sepals. Under the plant, 10–15 round tubers of yellowish-cream color weighing about 150 g are formed. The flesh of the tubers is white and gets slightly yellow during thermal processing.

It is an early-ripening table variety. The plant has a spreading, medium-tall, heavily foliated structure. The tubers are round-oval with a blunt tip, white smooth skin, small eyeholes, and white flesh. The potato shoots are characterized by rare berry formation. The flowers are white, medium-sized, and the sepals are strongly curved downward. The sprout is spherical with a red-violet base. Udacha potato variety stands out for its good yield and allows harvesting from 10 to 15, sometimes even up to 20 tubers weighing 100–150 grams from each healthy plant.

An early-maturing variety, which is excellent for harvesting and consumption in summer and autumn, intended for table use. It has versatile applications. Under optimal storage conditions, the storability of Udacha ranges from 88 % to 97 %. The tubers are not very susceptible to mechanical damage, making them convenient for commercial cultivation. The marketability is 96 % [20–21].

It is characterized by low susceptibility to diseases and is not affected by viral infections. It is low-maintenance. The ripening and yield of the potatoes are not dependent on weather conditions and soil types. Thanks to the fast formation of tubers, it attains marketable quality within 45 days after the emergence of the first sprouts. With proper care, it yields high harvests, which is 500 quintals per hectare.

The tubers were sprouted as follows: for the first 2–3 weeks, the tubers were sprouted in darkness at a temperature of +10–12 °C until the sprouts reached a length of 1–1.5 cm. Afterwards, the tubers were transferred to light (a bright room) and continued their sprouting for another 2 weeks at a temperature of +25 °C to obtain strong sprouts (green or violet in color).

Apical meristems, measuring 3–5 mm, isolated from the eyes of the tubers, were used.

In the beginning, the potato tubers were washed with a soapy solution in tap water for 5–10 minutes, and then rinsed in running tap water for about 15 minutes. All further manipulations were carried out under aseptic conditions in a laminar flow cabinet.

The explants were sterilized by the following methods:

1. The washed potato tubers and meristematic shoots were sterilized in a sterilizing solution: a commercial chlorine-containing reagent called bleach (2.8 % active chlorine sodium and 2.0 % hydroxide) diluted

with distilled water at a concentration of 3 % for 5–10 minutes. Afterwards, the shoots were rinsed with sterile distilled water for about 5–10 minutes, repeating the process 3–4 times.

2. The washed potato tubers and meristematic shoots were sterilized in a sterilizing solution: Tween 20 (*Tween 20*, a viscous liquid, polyoxymethylene sorbitan monolaurate), diluted with distilled water at a concentration of 10 % for 5–10 minutes. Then the shoots were rinsed with sterile distilled water for about 5 minutes, repeating the process 3–4 times.

3. The washed potato tubers and meristematic shoots were sterilized in a solution of Lyzoformin 3000 (glutaraldehyde, glyoxal, dodecyldimethyl ammonium chloride) diluted with distilled water at a concentration of 2 % for 5–10 minutes. Then the shoots were rinsed with sterile distilled water for about 5 minutes, repeating the process 3–4 times.

4. The washed potato tubers and meristematic shoots were sterilized in 96 % ethanol diluted with distilled water at a concentration of 2 % for 5–10 minutes. Then the shoots were rinsed with sterile distilled water for about 5 minutes, repeating the process 3–4 times.

All sterilized tubers of the potato varieties Aladin, Gala, Nevsky, Udacha, and Kostanay Novosti were transferred to growth stimulators such as Biostimulator, Epin, Heteroauxin, Kornevin and Humate. All stimulators were diluted with distilled water in a ratio of up to 250 ml and poured into sterile jars until the potato tubers were completely covered with the solution. All the jars containing the solutions and potato tubers were left in the laminar flow cabinet for 24 hours. For introduction into in vitro culture, the potato tuber pith was used, and the Murashige and Skoog nutrient medium was applied with 30 g/L sucrose, 4 g/L agar, pH 5.7 with added various concentrations of plant growth regulators (phytohormones) [22–24].

Results and Discussion

The efficiency of different options for sterilizing explants of different potato varieties using antimicrobial agents was compared and presented in Table 1.

Table 1

Efficiency of different sterilization options for explants of different potato varieties with the use of antimicrobial agents

Sterilizing Agents	Solution concentration	Number of infected explants, %									
		Gala		Udacha		Nevsky		Kostanay Novosti		Aladin	
		Exposure, min									
		5	10	5	10	5	10	5	10	5	10
Bleach	3 %	75 ±1.47	58 ±2.16	69 ±1.78	55 ±1.63	87 ±1.41	69 ±2.27	85 ±1.08	76 ±1.87	81 ±2.55	73 ±1.22
Tween	10 %	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00
Lyzoformin	2 %	100 ±0.00	97 ±1.78	100 ±0.00	98 ±1.87	100 ±0.00	96 ±2.16	100 ±0.00	98 ±1.87	100 ±0.00	97 ±1.47
Ethyl Alcohol	96 %	76 ±1.08	69 ±0.41	78 ±0.41	61 ±1.08	82 ±2.12	73 ±2.16	92 ±1.78	76 ±0.82	89 ±0.71	64 ±1.08
Note: The data in the table are presented as the arithmetic mean ± SD, $p<0.05$.											

The results of the conducted experiments showed that when using 10 % Tween and 2 % Lyzoformin as sterilizing agents, there was a 100 % contamination of the entire plant material. With an increase in exposure time to 2 % Lyzoformin by 10 minutes, the percentage of potato explants infection slightly decreased from 96 % to 97 %.

When using 96 % ethyl alcohol with an exposure time of 5 minutes, the number of infected explants ranged from 92 % to 73 %. With an increase in exposure time by 10 minutes in 96 % ethyl alcohol, the percentage of explant infection decreased significantly by 3.5 times.

The use of 3 % bleach for sterilizing the plant material resulted in a significant reduction in the percentage of infection in the potato explants. Increasing the sterilization timing with 3 % bleach to 10 minutes led to a 1.5 times reduction in potato explant infection and the complete absence of bacterial and fungal infections on the explants.

The choice of sterilizing agent directly affects the preservation of explants in a viable state (Table 2). The use of disinfecting agents, such as a solution of 2 % Lyzoformin and 10 % Tween with prolonged exposure (5 and 10 minutes) led to high death rates of shoots. At the same time, medium overgrowth was observed when using 10 % Tween solution: the Gala variety — 36 %, Udacha — 42.3 %, Nevsky — 68.2 %, Kostanay Novosti — 90.5 %, Aladin — 58.3 %. No medium overgrowth was observed when using the 2 % Lyzoformin solution. The treatment with the disinfecting agents, 2 % Lyzoformin and 10 % Tween solutions, did not significantly increase the number of sterile viable explants.

Table 2

Regeneration of explants after treatment with disinfectants (percentage of the total explants)

Variety Potato	Sterilizing agents, Solution concentration	Aseptic Viable Explants		Aseptic non-viable Explants		Infected Viable Explants		Infected non-viable Explants	
		Abs.	%	Abs.	%	Abs.	%	Abs.	%
Gala	Bleach 3 %	22	88	3	12	2	8	1	4
Udacha		24	92.3	2	7.7	3	11.5	2	7.7
Nevsky		19	86.4	3	13.6	2	9.1	1	4.5
Kostanay Novosti		18	87.7	3	14.3	4	19.1	2	9.5
Aladin		19	79.2	5	20.8	3	12.5	2	8.3
Gala	Twin 10 %	0	0	25	100	0	0	9	36
Udacha		0	0	26	100	0	0	11	42.3
Nevsky		0	0	22	100	0	0	15	68.2
Kostanay Novosti		0	0	21	100	0	0	19	90.5
Aladin		0	0	24	100	0	0	14	58.3
Gala	Lyzoformin 2 %	1	4	24	96	0	0	0	0
Udacha		2	7.7	24	92.3	0	0	0	0
Nevsky		2	9.1	20	90.9	0	0	0	0
Kostanay Novosti		1	4.8	20	95.2	0	0	0	0
Aladin		1	4.2	23	95.8	0	0	0	0
Gala	Ethanol 96 %	12	48	13	52	3	12	5	20
Udacha		13	50	13	50	4	15.4	6	23
Nevsky		9	41	13	59	2	9.1	5	22.7
Kostanay Novosti		11	52.4	10	47.6	2	9.5	4	19
Aladin		11	45.8	13	54.2	3	12.5	5	20.8
Note: The observed differences with potato varieties are statistically significant at $p < 0.05$.									

The use of bleach based on sodium hypochlorite for sterilization showed high viability of explants. However, in this case, there was also significant mortality of explants: Gala — 12 %, Udacha — 7.7 %, Nevsky — 13.6 %, Kostanay Novosti — 14.3 %, Aladin — 20.8 %.

During the growth of explants using the described treatment method, the development of infection was observed. Microorganisms grew on the surface of the agar layer around the explant, originating from the tissues and contaminating the nutrient medium. The infected viable explants showed the following percentages: Gala — 8 %, Udacha — 11.5 %, Nevsky– 9.1 %, Kostanay Novosti — 19.1 %, Aladin — 12.5 %.

When treated with 96 % ethyl alcohol compared to the bleach, the viability of explants was moderate: Gala — 48 %, Udacha– 50 %, Nevsky — 41 %, Kostanay Novosti — 52.4 %, Aladin — 45.8 %. The aseptic non-viable explants were observed in the experiment: Gala — 52 %, Udacha– 50 %, Nevsky– 49 %, Kostanay Novosti — 47.6 %, Aladin — 54.2 %.

At the same time, a number of viable explants with infection and a low yield of non-sterile explants ranged from 9.1 % to 15.4 %.

The laboratory experiment on the effect of sterilizing solutions on the yield of aseptic viable explants did not reveal significant differences between the potatoes varieties used.

The best result was obtained using the following procedure:

1. The washed potato tubers and meristematic shoots were sterilized in a sterilizing solution: a commercial chlorine-containing reagent — bleach (active chlorine — 2.8 %, sodium hydroxide — 2.0 %), diluted with distilled water to a concentration of 3 %, for 5–10 minutes. Afterwards, the shoots were rinsed with sterile distilled water for about 5–10 minutes, 3–4 times.

2. The washed potato tubers and meristematic shoots were sterilized with 96 % ethyl alcohol diluted with distilled water to a concentration of 2 %, for 5–10 minutes. Then, the shoots were rinsed with sterile distilled water for about 5 minutes, 3–4 times.

Conclusions

Thus, the obtained results indicate that the success of introducing meristematic potato shoots of the Aladin, Gala, Nevsky, Udacha, and Kostanay Novosti varieties into in vitro culture is influenced by the method of explant's sterilization.

During the laboratory experiments, it was found that the most effective sterilizing agents are 3 % bleach (Belizna) and 96 % ethyl alcohol with an exposure time of 10 minutes. Based on the experiment results, we propose using 3 % bleach as the main sterilizing agent with a 10-minute exposure time and for reducing surface contamination, preliminary treatment of explants with 96 % ethyl alcohol is recommended. The authors believe that 96 % ethyl alcohol is the most optimal option for sterilizing solutions since it is nontoxic, provides a gentle sterilization, and minimally damages the plant material tissues.

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Әртүрлі картоп сорттарының эксплантттарын *in vitro* жағдайында дақылға енгізу мақсатында оларды зарарсыздандыру жолын таңдау

Эксплант — өсімдік ұлпасының немесе органының фрагменті. Кез келген өсімдіктің ұлпасын *in vitro* жағдайда дақылға енгізу барысында инфекцияланбаған, тіршілікке қабілетті эксплантты таңдаудан басталады. Мақалада «Аладин», «Гала», «Невский», «Удача» және «Қостанай жаңалықтары» атты картоп сорттарын микроклоналды көбейту үшін олардың эксплантттарының тіршілікке қабілеттілігіне әртүрлі зарарсыздандыратын агенттердің әсері, сондай-ақ оларды өңдеу уақытын зерттеу нәтижелері көрсетілген. Бастапқы материал ретінде картоп түйнектерінен алынған меристемалық өскіндер болды. Белизна, Твин 20, Лизоформин 3000, 96 % этил спирті сияқты коммерциялық зарарсыздандыру агенттері қолданылған. Тәжірибе нәтижелері бойынша негізгі зарарсыздандыру агенті ретінде экспозиция уақыты 10 минут болатын 3 % Белизнаны қолдануды және беткейлік ластануын азайту үшін эксплантты 96 % этил спиртімен алдын ала өңдеу ұсынылған. Авторлар 96 % этил спирті ерітіндісін зарарсыздандырудың ең жақсы нұсқасы деп санайды, өйткені эксплантар үшін ерітінді улы емес, зарарсыздандырудың жұмсақ әдісі және өсімдік материалының тіндерін аз зақымдайды.

Кілт сөздер: картоп түйнектері, меристемалық өскіндер, зарарсыздандыратын ерітінділер, эксплант, экспозиция, зарарсыздандыру.

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Подбор условий стерилизации эксплантов различных сортов картофеля для введения в культуру *in vitro*

Эксплант — фрагмент ткани или органа растений, инкубируемый самостоятельно или используемый для получения первичного каллуса. Введение в культуру тканей *in vitro* любого растения начинается с подбора неинфицированного жизнеспособного экспланта. Авторами приведены результаты исследования влияния различных стерилизующих агентов, а также времени их обработки на жизнеспособность растительных эксплантов при микроклональном размножении картофеля сортов «Алладин», «Гала», «Невский», «Удача» и «Костанайские новости». Исходным материалом являлись меристемные ростки из клубней картофеля. Использованы такие коммерческие стерилизующие агенты, как Белизна, Твин 20, Лизоформин 3000, 96 %-ный этиловый спирт. Проанализировано влияние стерилизующих агентов, а также времени их обработки на жизнеспособность эксплантов и их контаминацию. В результате проведенных экспериментов было установлено, что наиболее эффективными являются 3 %-ная Белизна и 96 %-ный этиловый спирт с длительностью экспозиции 10 мин. По результатам эксперимента предлагаем в качестве основного стерилизующего агента использовать 3 %-ную Белизну со временем экспозиции 10 мин, а для уменьшения поверхностных загрязнений применять предварительную обработку эксплантов 96 %-ным этиловым спиртом. Авторы считают, что 96 %-ный этиловый спирт является наиболее оптимальным вариантом стерилизующих растворов, так как не токсичен, является щадящим способом стерилизации и минимально повреждает ткани растительного материала.

Ключевые слова: клубни картофеля, меристемные ростки, стерилизующие растворы, эксплант, экспозиция, стерилизация.

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