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Research Article

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Optimization of *In vitro* Sterilization and Initial Cultivation Methods for Local Garlic (*Allium sativum* L.) Varieties

This article explores the optimization of sterilization and initial cultivation methods for local garlic (*Allium sativum* L.) varieties under *in vitro* conditions. Due to the biological characteristics of garlic, which rarely produces viable seeds, and the limited efficiency of conventional vegetative propagation methods, planting material is often susceptible to the accumulation of viral and fungal infections. Therefore, the use of biotechnological approaches represents an important direction for improving plant health and producing high-quality, uniform planting material. In this study, explants of the "Arman", "Zailiysky", and "Dungansky" varieties were cultured on Murashige and Skoog (MS) nutrient medium. Effective sterilization methods were selected, and optimal conditions for growth were determined. According to the obtained results, all studied varieties demonstrated good morphogenetic responses and showed a high potential for micropropagation *in vitro*. This method enables the establishment of an *in vitro* collection of local garlic varieties and can be applied in agricultural production for obtaining high-quality planting material. Furthermore, this technology ensures biological safety and facilitates the rapid propagation of virus- and fungus-resistant forms of garlic.

Keywords: garlic, *in vitro*, micropropagation, explant, sterilization, collection.

Introduction

Garlic (*Allium sativum* L.) is one of the oldest agricultural crops, first cultivated in the countries of Central Asia [1]. It is considered one of the most widespread and important species of the *Allium* genus used both as food and for medicinal purposes worldwide [2]. Garlic is mainly propagated vegetatively; however, due to the difficulty in inducing flowering, its genetic improvement through breeding is limited [3]. Additionally, garlic bulbs cannot be stored for more than 6–8 months, requiring annual replanting. This process can become economically inefficient due to pest and pathogen infestations [4]. Prolonged storage in open fields leads to the accumulation of viral infections, resulting in reduced yield or complete crop loss [5]. Viral diseases of garlic cause significant yield losses globally [6], and several virus species can be transmitted throughout the plant's entire life cycle during vegetative propagation [7].

The absence of seed formation in garlic limits breeding and sanitation methods to clonal selection and meristem culture [8]. In recent years, *in vitro* techniques have been widely used for the conservation of rare and valuable plant species [9]. Virus elimination using meristem (shoot tips) culture has proven effective for vegetatively propagated crops, including garlic [10]. Clones obtained in this way exhibit higher productivity and improved quality [11], and tissue culture methods hold great potential for increasing yield and producing virus-free planting material [12]. Moreover, this method is also effective in genetic improvement, breeding, and conservation research on garlic [13]. For large-scale production of garlic bulbs, the use of meristem or shoot tips for micropropagation is more efficient compared to other explant sources [14].

The relevance of this topic is associated with the growing demand in agriculture for high-quality, disease- and pest-free planting materials, as well as the need to limit the spread of viral and fungal diseases. Micropropagation technology under *in vitro* conditions allows for the elimination of various biotic threats through the cultivation of explants in an aseptic environment.

The aim of this study is to establish an *in vitro* collection of domestic garlic (*Allium sativum*) varieties preserved in the genetic fund of the "Kazakh Research Institute of Fruit and Vegetable Growing" LLP and to determine effective methods for sterilization and initial cultivation. The theoretical and practical significance of the research lies in optimizing sterilization techniques for garlic explants and improving the composition

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of the nutrient medium using the *in vitro* method. By adapting the obtained plant samples to ex vitro conditions, it becomes possible to select disease-resistant and locally adapted forms, thereby expanding their potential use in agricultural production. Effective nutrient media and sterile conditions accelerate the microipropagaton of garlic and serve as a foundation for the development of seed production within the domestic biotechnology sector.

Micropropagation of garlic has been studied in many countries, and various combinations of nutrient media and growth regulators have been identified as effective. However, systematic studies on the *in vitro* cultivation and micropropagation of local garlic varieties have not previously been conducted in Kazakhstan. Therefore, the novelty of this study lies in the fact that, for the first time in Kazakhstan, the cultivation and adaptation characteristics of local garlic varieties under *in vitro* conditions have been investigated. The obtained results can serve as a basis for future selection of disease-resistant and high-yielding garlic forms**.**

The results of this research will contribute to the biotechnological modernization of garlic production in Kazakhstan, facilitate the production of high-quality planting material, and ensure stable agricultural productivity.

Experimental

The research was conducted in the biotechnology laboratory of the "Potato Breeding, Seed Production and Biotechnology" Department at the regional branch "Kainar" of the LLP "Kazakh Research Institute of Fruit and Vegetable Growing" (2024-2025).

As research objects, three domestic garlic (*Allium sativum*) varieties developed through the institute's breeding program were selected: "Arman", "Zailiyskiy", and "Dunganskiy".

Arman—a variety developed by the Kazakh Research Institute of Potato and Vegetable Growing, authored by Lakhin A.S. It is a mid-season, autumn-planted, bolting variety with a pungent taste, intended for general purposes. The bulbs are flat, white with a purple hue. On average, it has 10 large cloves weighing 6–9 g. It is disease-resistant with a yield of 6–10 t/ha. Realization is up 90 %, and the storage duration is 4-5 months. Since 1997, it has been recommended for cultivation in the Almaty region [15].

Zailiyskiy—a variety developed by Erenburg P.M. through mass selection from a local Dungan garlic population. It is a mid-season, autumn-planted, bolting variety. The bulbs are round-flat, white with a purple tint, weighing 35–55 g. It contains 5–8 cloves per bulb, each weighing 6–8 g. The average yield is 8–12 t/ha. It is resistant to cold and has a storage period of 4-5 months. Since 1955, it has been approved for cultivation in several regions [15].

Dunganskiy—a local Kazakh variety. It has a pungent flavor, is autumn-planted, and produces flower stalks. The growing season from sprouting to harvest is 120–130 days. The bulbs are round-flat with a purple tint and weigh 30–70 g. Each bulb has 5–9 cloves. The yield is 5.0–7.0 t/ha. Since 1959, it has been recommended for cultivation in the Zhambyl and South Kazakhstan regions [15].

The research was based on *in vitro* micropropagation methods. As explants, vegetative parts of garlic—specifically shoots derived from the root system—were used.

Sterilization procedures were carried out in several stages:

Pre-cleaning—Garlic samples were washed in a laundry soap solution and then rinsed 2-3 times in distilled water.

Chemical sterilization—Samples were treated in sodium hypochlorite solutions of varying concentrations for 2-3 minutes and then rinsed again 2-3 times in distilled water.

Aseptic processing—In a laminar flow cabinet, the outer scales of the garlic bulbs were removed, and shoots emerging from the root system were excised.

The explants were transferred into a pre-prepared nutrient medium. A modified Murashige and Skoog (MS) medium supplemented with phytohormones (0.5 mg/L indole-3-acetic acid(IAA) and 1.0 mg/Lbenzylaminopurine(BAP)) was used as the nutrient medium: MS salt base — 2.17 g; Sucrose — 15 g; Agar — 3.5 g; Thiamine HCl (Vitamin B1) — 0.5 mg/L; Pyridoxine HCl (Vitamin B6) — 0.25 mg/L; Total medium volume — 0.5 L.

After inoculation, the explants were placed in a phytotron room under controlled light conditions at a temperature of 25°C for cultivation.

Explants were cultivated in 50 mL glass test tubes, with one explant placed in each tube. As a nutrient medium, the basal Murashige and Skoog (MS) medium without the addition of phytohormones was used. The cultures were maintained under controlled phytotron conditions at 25 ± 2°C, with a 16-hour photoperiod

and 8 hours of darkness, and a light intensity of approximately 3000 lx. Each experimental variant included 30 explants and was conducted in three replications.

The obtained data were processed using Excel and Statistica 10.0 software. The results were presented as mean values ± standard error (SE), and the significance of differences was determined using Student's t-test and Duncan's multiple range test ($p < 0.05$).

Results and Discussion

During the study, three garlic cultivars—"Arman", "Zailiyskiy", and "Dunganskiy"—were cultivated under *in vitro* conditions. Effective sterilization methods were applied (Tab. 1), and all cultivars successfully adapted to the nutrient medium and began to grow without infection.

Table 1

Optimization of garlic explant sterilization methods

№	Garlic Variety	NaOCl Concentration, %	Treatment Time, minutes	Contamination Rate, %	Viability, %	Necrosis rate, %
1	"Arman"	0.5	3	40 ± 2.1*	50 ± 2.8	10 ± 1.5
		1.0	2	15 ± 1.2*	75 ± 3.0*	10 ± 1.3
		2.0	3	5 ± 0.8*	40 ± 2.5*	55 ± 3.2*
2	"Zailiyski"	0.5	3	35 ± 1.9*	55 ± 2.7*	10 ± 1.6
		1.0	2	10 ± 1.1*	80 ± 3.2*	10 ± 1.3
		2.0	3	8 ± 0.9	45 ± 2.1	47 ± 2.8*
3	"Dunganskiy"	0.5	3	38 ± 1.7*	48 ± 2.3	14 ± 1.9
		1.0	2	12 ± 1.0*	77 ± 3.1*	11 ± 1.4
		2.0	3	7 ± 0.8	43 ± 2.2	50 ± 2.7*

* – accuracy of differences in $p \leq 0.05$

As shown in Table 1, different concentrations of sodium hypochlorite (0.5 %, 1.0 %, and 2.0 %) and exposure times (2-3 min) were tested for garlic explant surface sterilization. The treatment with 1.0 % NaOCl for 2 minutes proved to be the most effective for all three varieties. Under these conditions, the contamination rate was low (10–15 %), the viability of explants was high (75–80 %), and the necrosis rate remained minimal (10–11 %). At a lower concentration (0.5 %), contamination levels increased (35–40 %) and viability decreased. Conversely, treatment with 2.0 % NaOCl reduced contamination but caused high toxicity, leading to a significant decrease in explant viability (40–45 %) and a marked increase in necrosis (up to 50 %). Therefore, sterilization with 1.0 % NaOCl for 2 minutes can be considered the optimal method for obtaining viable garlic explants under *in vitro* conditions.

Table 2

Growth performance of garlic varieties under *in vitro* conditions

№	Variety Name	Observation Time	Description	Shoot height, cm
Medium		**MS**		
1	"Arman"	On the 3 rd day	Aseptic, well adapted, initial sprouting observed	–
		On the 7 rd day	Active growth phase	1.4 ± 0.1
		On the 14 rd day	Root initiation and bud development observed	4.0 ± 0.2*
2	"Zailiyskiy"	On the 3 rd day	Explant adapted to the nutrient medium	–
		On the 7 rd day	Shoot formation and elongation started	1.5 ± 0.1
		On the 14 rd day	Active rooting and bud differentiation	5.0 ± 0.3*

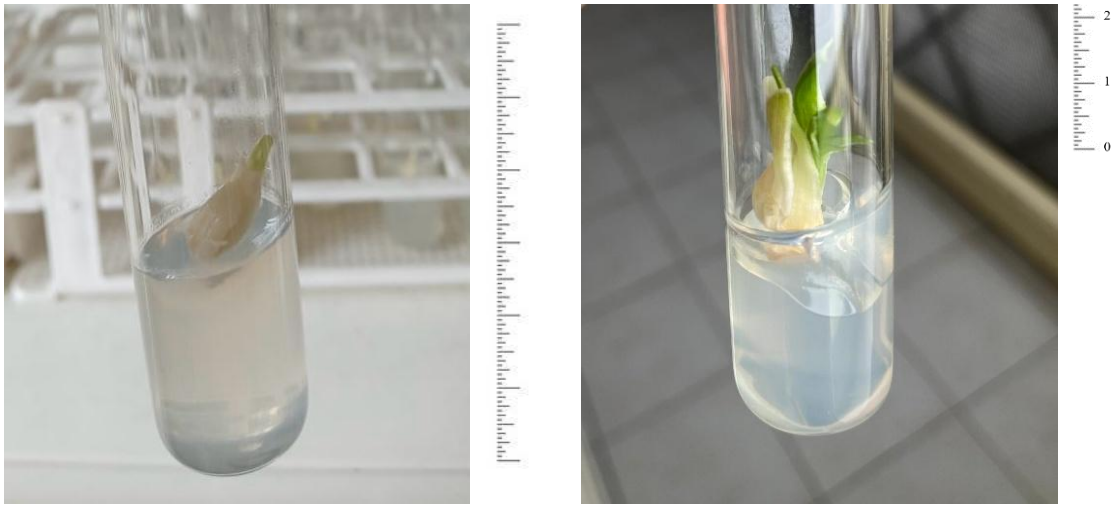
Continuation of Table 2

№	Variety Name	Observation Time	Description	Shoot height (cm)
Medium		**MS**		
3	“Dunganskiy”	On the 3 rd day	Explant adapted to the medium	–
		On the 7 rd day	Moderate growth activity observed	1.3 ± 0.1
		On the 14 rd day	Roots formed, buds clearly visible	4.0 ± 0.2*

* – accuracy of differences in $p \leq 0.05$

During the study, the adaptation, growth, and rooting processes of garlic explants were monitored on the 3rd, 7th, and 14th days of cultivation (Fig. 1). In the first three days, all varieties showed successful adaptation to the aseptic medium, with visible signs of sprouting. By the 7th day, the “Arman” variety entered the active growth phase (1.4 cm), while “Zailiyskiy” demonstrated slightly higher elongation (1.5 cm). The “Dunganskiy” variety exhibited a slower growth rate (1.3 cm). By the 14th day, all varieties displayed evident root and bud formation. The “Zailiyskiy” variety showed the highest shoot height (5.0 cm), whereas both “Arman” and “Dunganskiy” reached 4.0 cm. These findings indicate that the MS nutrient medium has a positive influence on the morphogenesis of garlic explants under *in vitro* conditions. Among the studied varieties, “Zailiyskiy” exhibited the best adaptation, vigorous growth, and strong rooting potential.

One of the important factors affecting plant morphogenesis under *in vitro* conditions is the mineral composition of the nutrient medium. According to Kalinin F.L. et al. [16], numerous studies have proposed various nutrient media depending on their mineral content. However, many researchers utilize different modified variants of the Murashige and Skoog (MS) medium for garlic propagation. The efficiency of clonal micropropagation largely depends on the proper selection of the nutrient medium. For *in vitro* clonal propagation of plants, researchers commonly employ media such as Murashige and Skoog (MS), Linsmaier and Skoog, Gamborg and Eveleg, Phillips, Heller, White, and Gautheret [17, 18]. Nonetheless, based on the outcomes of multiple experiments, it has been established that modified versions of the MS medium—with varied compositions of minerals, vitamins, cytokinins, and auxins—produce optimal effects for plant propagation. Successful *in vitro* introduction of plants also depends on the type of sterilizing agents used. The composition of sterilizing agents is selected according to the characteristics of the explant. The initial stage of the experiment involves the removal of saprophytic microflora from the plant explants before their introduction into *in virto* conditions, followed by placing them on nutrient media for growth [19].



a) After 3 days b) After 7 days

Figure 1. Monitoring of explants

The nutrient medium and growth conditions significantly influence the regeneration, rooting, and development of healthy plant forms from explants. This method plays an important role in selection, conservation of genetic resources, and identification of pathogen-resistant forms. According to the results of the study, monitoring of the garlic explants introduced into the *in vitro* nutrient medium after 14 days showed the initiation of root formation and the development of shoots up to 4-5 cm, as illustrated in Figure 2.

Figure 2. Observation of garlic explants after 14 days in *in vitro* conditions

According to global research experience, micropropagation of plants under *in vitro* conditions is used as a rapid and efficient method to multiply unique forms of specific cultivars, hybrids, and limited amounts of initial plant material. Compared to traditional vegetative propagation, the advantages of micropropagation include the high multiplication rate of desired plants regardless of the season. This method is widely applied for the conservation of gene pools of rare, endangered, and agriculturally valuable varieties of seeds, fruits, vegetables, and ornamental plants [20].

Conclusion

As a result of the study, the initial stages of *in vitro* cultivation of local garlic (*Allium sativum*) varieties were developed—including effective explant sterilization and optimization of primary growth conditions. The use of 1.0 % sodium hypochlorite for 2 minutes ensured the lowest contamination rate and the highest explant viability. The Murashige and Skoog (MS) nutrient medium had a positive effect on the initial growth and morphogenesis of garlic explants. Among the studied varieties, the "Zailiyskiy" variety demonstrated the highest growth activity and viability.

These results form an important foundation for improving the initial stage of *in vitro* micropropagation. Future studies will focus on developing an efficient method for micropropagation based on the obtained sterile plant material, optimizing the growth and rooting stages, as well as selecting disease-resistant forms and establishing a collection.

This research will be carried out at the "Kainar" regional branch of the LLP "Kazakh Research Institute of Fruit and Vegetable Growing". The obtained results will contribute to the biotechnological development of garlic cultivation in Kazakhstan and the production of high-quality, disease-resistant planting material.

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Conflict of interest

The authors declare no conflict of interest.

Author contribution

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript: **Matai Z.M.** — conceptualization, investigation, writing draft; **Zhantasov S.K.** — data curation, analysis, methodology, data collection; **Ibragimova G.M.** — investigation, data analysis.

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In vitro әдісі арқылы отандық сарымсақ (*Allium sativum L.*) сорттарын залалсыздандыру және бастапқы өсіру әдістерін оңтайландыру

Мақалада отандық сарымсақтың (*Allium sativum L.*) сорттарын *in vitro* жағдайында залалсыздандыру және бастапқы өсіру әдістерін оңтайландыру мәселесі қарастырылған. Сарымсақтың тұқым түзбейтін биологиялық ерекшелігі мен дәстүрлі көбейту тәсілдерінің шектеулі тиімділігі оны вирустық және саңырауқұлақты ауруларға бейім етеді. Сондықтан биотехнологиялық тәсілдерді қолдану өсімдіктерді сауықтыру мен сапалы, біркелкі отырғызу материалын алу үшін маңызды бағыт. Зерттеу барысында «Арман», «Заилийский» және «Дунганский» сорттарының эксплантты *Murashige & Skoog (MS)* қоректік ортасына енгізілді. Эксплантты залалсыздандырудың тиімді әдістері таңдалып, өсу процестеріне оңтайлы жағдайлар анықталды. Алынған нәтижелерге сәйкес, зерттелген сорттардың барлығы қоректік ортада жақсы морфогенез көрсетіп, микроклоналды көбейтуге бейімділік танытты. Бұл әдіс отандық сарымсақтың *in vitro* коллекциясын қалыптастыруға және ауыл шаруашылығында жоғары сапалы отырғызу материалын өндіруде қолдануға мүмкіндік береді. Сонымен қатар бұл технология өнімнің биологиялық қауіпсіздігін қамтамасыз етіп, вирустық және саңырауқұлақты ауруларға төзімді формаларды жедел көбейтуге жол ашады.

Кілт сөздер: сарымсақ, *in vitro*, микроклондау, эксплант, залалсыздандыру, коллекция

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Оптимизация методов стерилизации и первичного культивирования отечественных сортов чеснока (*Allium sativum L.*) методом *in vitro*

В данной статье рассматриваются вопросы оптимизации методов стерилизации и первичного культивирования отечественных сортов чеснока (*Allium sativum* L.) в условиях *in vitro*. Биологическая особенность чеснока — отсутствие семенного размножения, а также ограниченная эффективность традиционных методов вегетативного размножения — делает растение восприимчивым к вирусным и грибным заболеваниям. Поэтому применение биотехнологических подходов является важным направлением для оздоровления растений и получения качественного, однородного посадочного материала. В ходе исследования экспланты сортов «Арман», «Заилийский» и «Дунганский» были введены в питательную среду *Murashige & Skoog (MS)*. Были подобраны эффективные методы стерилизации эксплантов и определены оптимальные условия для их роста. Согласно полученным результатам, все исследованные сорта показали хорошую морфогенетическую активность и способность к микроклональному размножению в питательной среде. Разработанный метод позволяет создать *in vitro* коллекцию отечественных сортов чеснока и использовать её для производства высококачественного посадочного материала в сельском хозяйстве. Кроме того, данная технология обеспечивает биологическую безопасность продукции и открывает возможности для ускоренного размножения форм, устойчивых к вирусным и грибным заболеваниям.

Ключевые слова: чеснок, *in vitro*, микроклональное размножение, эксплант, стерилизация, коллекция

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