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Mitochondrial dysfunction in cellular senescence

Cellular senescence is a complex biological process characterized by cell cycle arrest and the loss of a cell's ability to divide. Despite the involvement of numerous molecular mechanisms, mitochondria play a central role in this process. Mitochondrial dysfunction, indicated by impaired respiratory capacity and a diminished energy status of the cell, is frequently accompanied by an augmented production of free oxygen radicals, resulting in oxidative stress. This condition not only accelerates cellular aging, but also its progression. A substantial body of research has substantiated the association between mitochondrial dysfunction and cellular senescence, underscoring the significance of mitochondria as a target for anti-aging therapies and interventions. The process of aging is associated with the onset of various age-related diseases, including cancer, cardiovascular diseases, and neurodegenerative diseases. A comprehensive understanding of these mechanisms offers novel opportunities to develop effective strategies that can mitigate the effects of senescence. This article summarizes the mechanisms contributing to the development of mitochondrial dysfunction during aging and discusses the main consequences of this impairment, particularly in the context of its impact on cellular senescence.

Keywords: cellular senescence, mitochondria, oxidative stress, mitophagy, microRNAs, age-associated diseases, cancer, Alzheimer's disease, cardiovascular diseases.

Introduction

Cellular senescence is an inevitable state of cells caused by various stress effects and physiological processes, which is characterized by irreversible cell cycle arrest. The process of senescence is influenced by numerous factors, including the accumulation of DNA damage, the shortening of telomeres, and the deterioration of mitochondria [1]. Senescence cells are known to accumulate in the body, which has been associated with an increased risk of developing multiple chronic diseases, decreased physical stability, and mortality [2]. The precise etiology of aging remains to be elucidated, rendering it a fundamental subject in contemporary scientific inquiry.

Mitochondria are membrane-bound organelles that play a central role in the energy metabolism of cells. They are responsible for a significant part of the production of adenosine triphosphate (ATP) molecules required for various cellular processes. In recent years, mounting evidence has indicated a causal relationship between mitochondrial dysfunction and the major mechanisms of senescence. The process of senescence itself has been demonstrated to be a significant factor in the development of various age-related diseases, including cancer, neurodegenerative diseases, and cardiovascular disease [3]. The exploration of these mechanisms may yield novel strategies for decelerating the aging process and addressing age-related diseases. This review aims to explore the intricate relationship between mitochondria and the senescence process, emphasizing the pivotal role of mitochondria in the development of age-related diseases.

Mitochondria and their role in senescence processes

The efficient operation of mitochondria is vital for the normal functioning of the organism. Mitochondria can be regarded as highly dynamic structures, capable of rapid and substantial adjustment to conditions that reflect the needs of cells. However, it is important to note that various environmental factors, including radiation exposure, as well as endogenous agents, have the capacity to exert influence on mitochondrial function [4, 5]. For example, the synthesis of antioxidants is augmented under conditions of elevated oxidative stress, as well as during periods of enhanced physical exertion, resulting in an escalation of the main mitochondrial enzymes present within skeletal muscle tissues [6]. However, exposure to these factors can lead to mutations in mitochondrial DNA, which in turn contributes to impaired mitochondrial function. Disruption of mitochondrial integrity, both structurally and functionally, is a primary factor contributing to accelerated cellular senescence [4, 7–9].

A plethora of theories has been postulated regarding the etiology of aging. Presently, the free-radical theory of aging, initially proposed by Denham Harman in the mid-1950s [10], occupies a central position among these theories. This theory posits that the accumulation of mitochondrial damage leads to the formation of free radicals, which, when present in excess, can damage cellular components such as proteins, lipids, and nucleic acids.

Mitochondrial DNA (mtDNA) has been shown to be more prone to damage in comparison to nuclear DNA. Its mutagenesis rate has been observed to be 10–20 times higher [11]. This heightened susceptibility can be attributed to the absence of histones and introns within the mtDNA genome, the inefficacy of repair mechanisms, and the proximity to sites of reactive oxygen species formation [12, 13]. It has been established that the frequency of mitochondrial DNA mutations increases with age, and excessive amounts of these mutations can disrupt the normal operation of mitochondria, leading to their dysfunction [14, 15].

Mitochondria are responsible for synthesizing approximately 90 % of the cell's energy, thus acting as the cell's multifunctional energy source. They synthesize ATP in a process known as oxidative phosphorylation via five electron-transport chain complexes. Mutations in mtDNA, which affect genes involved in oxidative phosphorylation, have been shown to have deleterious consequences for cellular energy metabolism, including increased free radical production and decreased antioxidant defenses in the body [16, 17]. Oxidative stress, characterized by an imbalance between the generation and elimination of free radicals, leading to their excessive formation, is considered a contributing factor to cellular senescence. Indeed, research has demonstrated that senescent cells exhibit reduced ATP production efficiency and a diminished energy status [18–20].

Mitochondria, being semi-autonomous organelles, possess a variety of mechanisms that enable them to maintain their integrity under stress. Mitophagy, a term derived from the Greek for "cell death", is a critical process involved in maintaining a healthy mitochondrial population through the constant destruction of dysfunctional mitochondria [21].

The degradation process is mediated by the autophagosome and was first demonstrated in mammalian cells by electron microscopy [22]. The most studied mitophagy pathway is the Parkin/PINK1-mediated pathway [23]. For example, overexpression of PTEN-induced kinase 1 (PINK-1) in dopaminergic neurons extends lifespan in drosophilia, whereas loss of Parkin shortens lifespan [24]. By scavenging dysfunctional mitochondria, mitophagy prevents excessive release of damage-associated molecular patterns (DAMPs), which are based on free-circulating mtDNA [25]. If the damaged mitochondrion cannot be neutralized, mitochondrial membrane rupture and cytosolic release of DAMPs occur, leading to strong inflammatory responses.

Proper control of mitochondrial function can limit inflammation and preserve cell function during senescence, which is supported by numerous experiments [26–28]. In one study, mitophagy also positively affected skeletal muscle cells in mice and humans, preventing senescence [28]. In contrast, disruption of mitophagy contributed to accelerated senescence and the development of several human diseases, including age-related diseases such as Parkinson's disease, Alzheimer's disease [29], cardiovascular disease, and cancer [30, 31].

Mitochondria were originally thought to be isolated organelles, but there is now increasing evidence that they are constantly undergoing fusion and fission. Together, these processes form the basis of mitochondrial dynamics and may also be involved in the control and elimination of dysfunctional mitochondria [32]. The major proteins that enable these processes are mitofusin 1 and mitofusin 2 (Mfn1 and Mfn2) [33]. Mitochondrial fusion can facilitate the exchange of mitochondrial components, including the exchange of mtDNA, which allows the replacement of missing or damaged components in the mitochondrial network [34]. In another case, the mitochondrial proteins Mfn1 and Mfn2 can be degraded, depending on the Parkin-dependent mitophagy pathway, to prevent the fusion of damaged mitochondria with the healthy mitochondrial network [35].

Fission is also a key process in maintaining a healthy mitochondrial population, separating damaged mitochondria from the overall network. Mitochondrial dysfunction disrupts these processes, which can result in a fragmented network dominated by small round or elongated mitochondria [36]. In senescent cells, mitochondria tend to be in a hyper-split state, and deficits in their integrity may contribute to the initiation of various diseases [37, 38], as well as accelerated cellular senescence [39, 40].

In addition to reduced energy status, mitochondrial dysfunction is associated with the development of chronic inflammation [41]. Inflammation is a hallmark of senescence and a risk factor for the development of several diseases, negatively affects the immune system, and accelerates cellular senescence [42, 43]. DAMPs

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are the major triggers of inflammation. They are recognized by specialized receptors of the innate immune system, such as toll-like receptors (TLRs) [44, 45]. Activation of this receptor initiates signal transduction pathways that normally trigger inflammation, resulting in the production of pro-inflammatory cytokines, particularly tumor necrosis factor TNF-α and interleukins (IL-6, 8, 12) [46].

Several studies have confirmed a correlation between levels of free circulating mtDNA in cells and proinflammatory cytokines, suggesting a link between mitochondrial dysfunction and inflammatory status [47, 48]. It is now well established that chronic inflammation underlies several age-related diseases such as atherosclerosis, Alzheimer's disease, Parkinson's disease, and type 2 diabetes [49–51].

After reviewing the major functions of mitochondria, we can conclude that they are important regulators of both energy and inflammatory processes, making them key players in the mechanisms of senescence and the pathogenesis of various diseases. As we have seen, genetics plays an important role in controlling and modulating mitochondrial functions. However, in addition to genetics, there is another important aspect that deserves attention — mitochondrial microRNAs.

Mitochondrial microRNAs and their involvement in cellular senescence processes

MicroRNAs (miRNAs) are a group of small non-coding RNAs that play an important role in the regulation of gene expression. The fact that microRNAs are located in mitochondria was discovered only recently, with the first discovery made by Barray and colleagues in 2011 [52]. These molecules have been collectively named "MitomiR", and each mitochondrion has its own unique set of microRNAs specific to a particular cell type [53]. The origin of these elements may be either the nuclear or mitochondrial genome, and their function is of paramount importance in ensuring proper mitochondrial functionality. They fulfill this role by regulating mitochondrial genes themselves or by modulating the expression of nuclear genes that play a role in mitochondrial processes [54, 55]. In addition, mitochondrial microRNAs can influence mitochondrial dysfunction, making them one of the major catalysts for accelerated cellular senescence [56–58].

Recent studies show that MitomiR serves as crucial sensors of cellular senescence, exerting control over mitochondrial homeostasis and influencing metabolic state, redox balance, apoptosis, mitophagy, all processes closely related to senescence. Some mitochondrial microRNAs, associated with cellular senescence are summarized in the Table.

T a b l e

The role of mitochondrial microRNAs in senescence

Name	Level	Role in senescence	Reference
miR-15b:	↓	Promotes formation of mitochondrial ROS, decreases mitochondrial membrane potential. Causes ATP deficiency, impairing cellular metabolism. Causes the development of a senescence-associated secretory phenotype (SASP), accelerating cellular senescence.	[59]
miR-181c:	↓	Disrupts respiratory complex IV, causing mitochondrial dysfunction. Enhances cellular damage through excessive production of ROS.	[60]
miR-4485:	↑	Negatively modulates respiratory complex I activity, ATP production, increases ROS levels. Inhibits caspase-3/7 activation and apoptosis, slows down mitophagy.	[61]
miR-181a, miR- 34a:	↑	Activate Bcl-2, affecting sensitivity to apoptosis, leading to impaired mitophagy, accumulation of old cells. Contribute to mitochondrial dysfunction.	[56]
miR-146a-5p:	↑	Promotes activation of NF-κB pathway, initiates transcription of pro-inflammatory cytokines. Accelerates senescence through SASPs.	[62]
miR-1, miR-133a, let-7b:	↓	Negatively modulates the function of key proteins involved in oxidative phosphorylation, reduce ETC functionality. Decrease the energy status of the cell.	[62]
miR-378-3p:	↑	Decrease the functionality of ATP synthase. Decrease oxidative capacity.	[62]

Continuation of Table

Name	Level	Role in senescence	Reference
miR-20b, miR-214, miR-200a-3p:	↑	Negatively affect the expression of MFN1 and MFN2 genes, Disrupt the mitochondrial fusion-release balance, leading to impaired fusion and increased fragmentation.	[63–65]
miR-17:	↓	Promotes ROS formation.	[66]
miR-574-5p:	↑	Regulates protein expression of mitochondrial electron transport chain (ETC) genes, supporting normal mitochondrial function.	[67]
miR-762:	↑	May contribute to inhibition of ATP production and induction of ROS formation and apoptotic cell death.	[68]
miR-106a:	↑	Negatively regulates the expression of some critical cell cycle and apoptosis factors. Inhibits mitophagy.	[66, 69]

Thus, it is easy to speculate that miRNAs can regulate mitochondrial function, and this phenomenon has important implications for the aging process, as mitochondrial dysfunction has devastating consequences for cell fate.

Mitochondrial Dysfunction and Age-Related Diseases

Neurodegenerative diseases

To date, the pathogenesis of neurodegenerative diseases remains in the center of scientific attention as it represents one of the most important problems facing modern society [70]. The factors underlying cognitive impairment in both natural aging and neurodegenerative diseases are not fully understood. There is considerable evidence that mutations in mitochondrial DNA and oxidative stress contribute to accelerated cellular senescence, which is a major risk factor for neurodegenerative diseases.

Alzheimer's disease (AD) is the most common neurodegenerative disease characterized by impairment of memory, language, and other thinking skills, with dementia at its core [70, 71]. A number of mitochondrial abnormalities have been identified in AD: changes in mitochondrial structure, mutations in mtDNA, changes in mitochondrial membrane potential, formation of ROS, decreased ATP, and impaired mitochondrial fusion. There is a wealth of evidence linking mtDNA mutations to the pathogenesis of AD [72–78].

The brain is particularly susceptible to oxidative damage due to its high oxygen consumption. One of the most common defects in the mitochondrial ETC in AD is cytochrome-c-oxidase deficiency, which leads to increased production of ROS and impaired energy metabolism [79]. Several studies have shown impairments of all five ETC complexes in different brain regions in AD [80]. Numerous studies have documented that mitochondrial dysfunction due to abnormal ROS processing is an important factor in the pathogenesis of Alzheimer's disease [81]. It is known that brain cells must constantly produce ATP to maintain neuronal function. For example, oxidative damage to the promoter of the gene encoding a subunit of mitochondrial ATP synthase can lead to a decrease in its level, resulting in decreased ATP production, increased oxidative stress, and cell death [82, 83]

Oxidative stress may also contribute to the pathogenesis of AD by disrupting calcium homeostasis [84, 85]. Glutamate is a neurotransmitter in the mammalian central nervous system that often mediates the synaptic transmission of nerve impulses. However, high levels of glutamate can be toxic, promoting neuronal death [86], and dendritic degeneration [87, 88]. Increased extracellular glutamate leads to its binding to NMDA calcium receptors. NMDA activation causes a massive influx of sodium and calcium into neurons and an outflow of potassium. Elevated Ca2+ levels cause irreversible damage to neurons, which promotes neuronal death [89].

Mitochondrial dysfunction and glutamate toxicity are linked. It has been shown that neurons have the ability to "burn" glutamate in the mitochondria, thereby preventing its toxicity [90]. In one of the studies, it was observed that when pyruvate is inhibited in neurons, glutamate consumption by the neurons increases, which leads to a decrease in extracellular glutamate levels, resulting in a decrease in cell death [91]. It is logical to assume that mitochondrial dysfunction will inhibit this process, resulting in an opposite increase in extracellular glutamate levels. Thus, neuroinflammation caused by mitochondrial dysfunction leads to neuronal loss and impaired neuronal plasticity, ultimately leading to Alzheimer's disease Mitophagy is a critical pathway for mitochondrial quality control. The accumulation of beta-amyloid (Aβ) and phosphorylated tau-protein (pTau) in the brain is a pathological hallmark of Alzheimer's disease. Aβ and pTau impair mitochondrial integrity and exacerbate mitochondrial dysfunction. Oxidative damage caused by Aβ and pTau leads to

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decreased levels of PINK1 and Parkin protein, which inhibits mitochondrial autophagy and thereby increases Aβ and pTau [92]. Disruption of fission or fusion processes, namely mutations in the MFN1, MFN2, and OPA1 genes, are also found in several neurodegenerative diseases, including AD [93–96]. In addition, abnormal mitochondrial fission and decreased expression of proteins related to their biogenesis (PGC-1α, TFAM, and NRF2) have been observed in AD patients, indicating impaired mitochondrial dynamics and biogenesis [97].

Parkinson's disease (PD) is a common neurodegenerative disorder associated with motor dysfunction. A loss-of-function mutation in PARK2 is the most common cause of early-onset PD [98–100]. Loss of function of the PINK1 gene, which encodes a mitochondrial serine/threonine kinase, is the second most common cause of PD [101,102].

Cancer

As early as 1924, Heinrich Warburg discovered that tumor cells are characterized by high glucose consumption and use "aerobic glycolysis" to produce ATP even when oxygen is available. Based on these observations, it has been suggested that altered respiratory capacity caused by mitochondrial abnormalities may be one of the causes of cancer development. Indeed, increased glucose uptake and decreased OXPHOS activity have been observed in many tumor types, and it is believed that high glycolytic capacity is an important hallmark of cancer. For example, glycolysis is common in rapidly growing tumors and oxidative phosphorylation is slowed in these tumors [103].

Oxidative stress due to mitochondrial dysfunction, which is characterized by the production of reactive oxygen species in cells, plays a critical role in cancer development by affecting genome stability and signaling pathways in the cellular microenvironment. Large amounts of ROS, which are by-products of mitochondrial dysfunction, are known to irreversibly damage cellular components, including nucleic acids. Such damage can cause genetic or epigenetic alterations by upregulating oncogenes and tumor suppressor genes. For example, impaired expression of the gene encoding NADH dehydrogenase can stimulate aerobic glycolysis, ROS production, and tumor growth [104].

In addition, ROS can activate various signaling pathways that may contribute to oncogenesis. Examples include the epidermal growth factor receptor EGFR signaling pathway or the Akt/NF-κB-dependent signaling pathway, which correlate with cancer development [105, 106].

IDH is a family of enzymes involved in oxidative phosphorylation. It includes three isoforms located in the cytoplasm, peroxisomes (IDH1) and mitochondria (IDH2 and IDH3). Some studies show that many tumors, including gliomas and leukemias, have mutations in the genes encoding IDH1 or IDH2 [107, 108].

Cardiovascular Diseases

One of the leading causes of death worldwide is cardiovascular disease (CVD). CVDs are a group of multifactorial diseases that affect the heart or blood vessels. Heart cells have a high energy demand, requiring a constant supply of ATP to maintain cardiac activity. In cardiomyocytes, mitochondria make up about one-third of the cell volume. Not surprisingly, proper mitochondrial function and dynamics are critical for these cells, and their dysfunction is a key factor in cardiovascular disease.

Oxidative stress is central to the development of CVD. Reduced mitochondrial function leads to the production of reactive oxygen species, depletion of cellular ATP, cellular damage and cardiomyocyte apoptosis. Unregulated production of ROS is responsible for a variety of cardiovascular diseases, including cardiac hypertrophy, heart failure, and cardiac ischemia-reperfusion injury [109].

Alterations in mtDNA genes, including NADH dehydrogenase, cytochrome b, and ATP synthase genes, are observed in cardiomyopathies and heart failure [110]. In heart failure, mitochondria are damaged by membrane rupture and depletion of their matrix, resulting in decreased ATP synthesis [111]. It has been shown that patients with heart failure have decreased activity of respiratory complexes I and IV [112]. A study has also shown that the process of mtDNA replication is impaired in cardiomyocytes from people with heart failure, resulting in depletion of mitochondrial DNA, reduction of mitochondrial proteins, and impaired mitochondrial biogenesis [113].

A study has also shown that the process of mtDNA replication is impaired in cardiomyocytes from people with heart failure, resulting in depletion of mitochondrial DNA, reduction of mitochondrial proteins, and impaired mitochondrial biogenesis [114]. Cardiac ischemia due to oxygen deprivation leads to mitochondrial fragmentation due to dysregulation of the Mfn2 protein [115]. The role of mitophagy in the development of CVDs cannot be overlooked. Atherosclerosis is an inflammatory disease of the arteries associated with im-

paired lipid metabolism. The pathogenesis of this disease is associated with the accumulation of macrophages, lipids, cholesterol, migration and proliferation of vascular smooth muscle cells. Mitophagy plays a key role in the removal of these accumulated substances, and disruption of this process will have devastating consequences, increasing vascular plaque formation due to the increase in ROS from damaged mitochondria [116].

Prospects for using mitochondria to fight senescence

Given the close association of mitochondria with senescence, several approaches have been developed and used as strategies to treat mitochondrial dysfunction and age-related diseases. Mitochondrial transplantation is one of the new therapeutic methods used to treat age-related diseases, especially cardiovascular diseases. The essence of the method is the transfer of "healthy" donor mitochondria into cells to replace dysfunctional or damaged mitochondria.

The concept of transferring mitochondria between cells is similar to installing new batteries in malfunctioning devices. By providing healthy mitochondria to cells with impaired energy metabolism, their ability to produce ATP and maintain cell survival can be restored.

One study showed that mesenchymal stem cells could gradually transfer their mitochondria to lung epithelial cells through structures called tunneled nanotubes (TNTs). This transfer helped reduce ATP loss in BEAS-2B cells exposed to cigarette smoke [117]. In another study, umbilical cord mesenchymal stem cells successfully transferred their mitochondria into mtDNA-deficient cells. This restored the expression of genes encoding mitochondrial proteins and improved the function of the ETC [118].

The energy produced by transplanted mitochondria can improve the function of recipient cells. For example, transplantation of isolated mitochondria into ischemic hearts helps to reduce infarct size and improve senolytic function [119]. Mitochondrial transfer opens new doors by providing additional options for the treatment of age-related diseases.

In recent years, hay therapy targeting mitochondria and antioxidants has gained particular popularity. Senotherapy is divided into two groups: senolytics, which kill senescent cells, and senomorphics, which inhibit inflammation. Senolytics have been shown to be highly effective in treating a wide range of age-related diseases, and clinical trials are currently underway for many of these modalities.

Examples of senolytic drugs include BH3 mimetics such as ABT-263 (navitoclax). These drugs are used in certain types of senescent cells based on increased expression of anti-apoptotic proteins of the BCL-2 family [120]. By inhibiting anti-apoptotic proteins, these drugs stabilize mitophagy and thereby remove old cells. In a study, procyanidin, a component of grape seed extract, was shown to have senotherapeutic activity and to extend the lifespan of mice by inhibiting SASP expression [121].

Thus, the use of mitochondria in the fight against aging represents a promising direction that opens new opportunities for improving health and quality of life. The development of various therapeutic approaches aimed at restoring mitochondrial function and overcoming age-related changes may have a significant impact on the treatment of age-related diseases. These strategies highlight the importance of mitochondria as key players in the aging process and their potential role in the future medical approach to longevity.

Conclusion

This article has reviewed the multifunctional role of mitochondria in senescence processes and their impact on cellular physiology. As the major source of cellular energy, mitochondria act as key regulators of oxidative stress in cells. Various environmental factors affect mitochondrial function on a daily basis, leading to an increased frequency of mitochondrial DNA mutations. All of this leads to mitochondrial dysfunction, which in turn causes a decrease in ATP synthesis, production of reactive oxygen species, and promotes chronic inflammation. The vicious cycle between oxidative stress and mitochondrial dysfunction leads to the development of cellular senescence. Furthermore, microRNAs have been demonstrated to play a pivotal role in the regulation of mitochondrial function, which is a critical aspect in comprehending the mechanisms of cellular senescence. A variety of age-related pathologies are marked by distinct mitochondrial mutations, which may contribute to the development and progression of these diseases. Consequently, mitochondria emerge as pivotal subjects for research in the biology of aging and the development of novel therapeutic strategies. A comprehensive understanding of these mechanisms could lead to the development of interventions that enhance health in older individuals and decelerate the senescence process at the cellular level.

Author Contributions

The manuscript is a review article prepared through the joint contribution of all authors. All authors have read and approved the final version of the manuscript. CRediT: **Solomko L.R.** – Conceptualization, Literature collection and analysis, Writing – Original Draft. **Kussainova A.A.** – Methodology, Literature collection and analysis, Writing – Review & Editing, Visualization. **Bulgakova O.V.** – Data Curation, Writing – Review & Editing, Critical analysis and Final text preparation.

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Жасушаның қартаюы кезіндегі митохондриялық дисфункция

Жасушаның қартаюы — жасушалық циклдің тоқтауымен және жасушаның бөліну қабілетінің жоғалуымен сипатталатын күрделі процесс. Қартаю көптеген молекулалық механизмдерден туындаса да, оның дамуында митохондриялар маңызды орын алады. Тыныс алу қабілетінің бұзылуымен және жасушаның энергетикалық статусының төмендеуімен көрінетін митохондриялық дисфункция жиі тотығу стресіне әкелетін бос оттегі радикалдарының жоғарылауымен бірге жүреді. Бұл жағдай жасушаның қартаюына ықпал етіп қана қоймайды, сонымен қатар оның дамуын тездетеді. Бүгінгі күні митохондриялық дисфункция мен жасушалық қартаю арасындағы байланысты растайтын көптеген зерттеулер бар, бұл митохондриялардың қартаюға қарсы терапия мен араласудың мақсаты ретіндегі маңыздылығын көрсетеді. Қартаю әртүрлі жасқа байланысты аурулар мен жағдайлардың, соның ішінде қатерлі ісік, нейродегенеративті аурулар және жүрек-тамыр ауруларының пайда болуымен байланысты. Бұл механизмдерді түсіну қартаюды бәсеңдетуге бағытталған стратегияларды әзірлеу үшін жаңа көкжиектерді ашады. Мақалада қартаю процесінде митохондриялық дисфункцияның дамуына ықпал ететін механизмдер жинақталған, сонымен қатар бұл бұзылыстың негізгі салдары, әсіресе жасушалық қартаюға әсер ету контексінде қарастырылған.

Кілт сөздер: жасушаның қартаюы, митохондрия, тотығу стресі, митофагия, микроРНҚ, жасқа байланысты аурулар, қатерлі ісік, Альцгеймер ауруы, жүрек-қан тамырлары аурулары.

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Митохондриальная дисфункция при клеточном старении

Клеточное старение — это сложный биологический процесс, который характеризуется остановкой клеточного цикла и утратой способности клеток к делению. Несмотря на участие множества молекулярных механизмов, центральную роль в этом процессе играют митохондрии. Митохондриальная дисфункция, проявляющаяся нарушением дыхательной способности и снижением энергетического статуса клетки, часто сопровождается увеличением продукции свободных радикалов кислорода, что ведет к окислительному стрессу. Это состояние не только способствует клеточному старению, но и ускоряет его прогрессирование. На сегодняшний день существует множество исследований, подтверждающих связь между митохондриальной дисфункцией и клеточным старением, что подчеркивает важность митохондрий как мишени для антивозрастных терапий и вмешательств. Старение связано с возникновением различных возрастных заболеваний и состояний, включая рак, нейродегенеративные и сердечно-сосудистые заболевания. Понимание этих механизмов открывает новые горизонты для разработки стратегий, направленных на замедление старения. В данной статье обобщены механизмы, способствующие развитию митохондриальной дисфункции в процессе старения, а также рассмотрены основные последствия этого нарушения, особенно в контексте влияния на клеточное старение.

Ключевые слова: клеточное старение, митохондрии, окислительный стресс, митофагия, микроРНК, возраст-ассоциированные заболевания, рак, Болезнь Альцгеймера, сердечно-сосудистые заболевания.

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Species Composition of Blood-Sucking Mosquitoes of the Genus *Aedes (Diptera: Culicidae)* in the Karaganda Region

This study examines the species composition of blood-sucking mosquitoes in the Karaganda Region of Central Kazakhstan — a region where faunal and ecological data have been scarce. Through field surveys and morphological identification, we documented at least ten species belonging to the genus *Aedes* Meigen, 1818. Notably, several specimens exhibited atypical morphological traits, suggesting the presence of previously unrecognized or cryptic forms within local populations. These findings underscore the importance of integrative taxonomic approaches — particularly molecular methods — for clarifying the status of morphologically distinct variants and improving our understanding of their epidemiological relevance. Community-level analyses of species diversity and evenness revealed pronounced spatial differences between localities: The species *Ae. (Och.) dorsalis* Meigen, 1830 was dominant in and around Karaganda City, whereas *Ae. (Rus.) subdiversus* Martini, 1926 was more prevalent in the Nura District. The detection of species not previously reported from the region further suggests a potential range expansion of *Aedes* mosquitoes. Collectively, these results provide the first systematic baseline for *Aedes* biodiversity in Central Kazakhstan and underscore its importance for vector ecology, arbovirus transmission risk, and public health surveillance in the region.

Keywords: Aedes, *Culicidae*, insecta, species, Shannon index, Pielou's evenness index, Jaccard index, fauna.

Introduction

Aedes (Diptera: Culicidae) is a genus of mosquitoes that includes the largest number of species of significant medical importance, as they serve as vectors for a variety of viral pathogens. Notably, they are known to transmit the deadly viruses of Dengue, Zika, Chikungunya, and West Nile fever [1]. Mosquitoes of the genus Aedes Meigen, 1818 are found in various regions of the Republic of Kazakhstan, with their abundance and species composition varying significantly depending on the local climate and landscape. One of the least studied regions of Kazakhstan in terms of the species diversity of blood-sucking mosquitoes of this genus is the Karaganda Region, located in Central Kazakhstan.

In recent decades, certain mosquito species have expanded their range into parts of Europe, Russia, and the North America due to climatic and environmental changes. These include *Ae. (Stegomyia) albopictus* Skuse, 1894, *Ae. koreicus* Edwards, 1917, and *Ae. japonicus* Theobald, 1901. Intercontinental spread is primarily the result of egg transportation attached to various plant products and tires shipped via cargo vessels, which are of particular relevance [2]. Air travel also plays a significant role in their dispersal [3]. However, on national and regional scales, ground transportation — particularly by cars and trains — is considered the most important route of spread [4]. For instance, the invasive mosquito *Ae. koreicus*, a Far Eastern species, has been detected within the territory of the Republic of Kazakhstan [5]. Reports of this species have become increasingly common in Europe, Russia Far East, and the North Americas [6]. As the ranges of mosquito vectors expand, so too do the distributions of the pathogens they transmit. While the potential for future arbovirus transmission by invasive mosquito species in these regions is concerning, circulation of several arboviruses has already been observed among native species. Notably, three such viruses include Sindbis virus, Usutu virus (USUV), and West Nile virus (WNV) [7].

The aim of our study is to clarify the species composition of g. *Aedes* mosquitoes in the Karaganda Region of Kazakhstan.

Experimental

To study the species composition of g. *Aedes* mosquitoes, larval samples of blood-sucking mosquitoes were collected in the Karaganda Region (Fig. 1). Larvae were collected (Table 1) using the methods described by Monchadskij (1952) [8], employing a specialized dipping tray and a pear-shaped pipette. The collected larvae were preserved in 5 ml tubes containing 96 % ethanol or Carnoy's solution for cytogenetic analysis. Species identification was carried out using morphological methods based on the works of Gucevich et al. (1970) [9], Dubitsky [10], and Becker (2010) [11].

Samples were divided according to geographic origin. The first zone included specimens collected in Karaganda city and adjacent areas. The second zone comprised samples from the Nura District, located in the southwest of the Karaganda Region, approximately 200 km from the regional center (Table 3). Larvae were collected from water bodies formed by melting snow.

Table 1

***Aedes* Larvae Sampling Sites within the Karaganda Region**

No.	Location of Temporary and Permanent Water Bodies (Flood Areas)	Sampling Date	Number of Larvae	Coordinates
Zone I				
1	Karaganda, Yugo-Vostok Microdistrict	24.04.24	65	49.77816 N, 73.1631 E
2	Karaganda, Yugo-Vostok Microdistrict	24.04.24	50	49.7577 N, 73.15977 E
3	Karaganda, Kungey Microdistrict	24.04.24	82	49.77806 N, 73.16301 E
4	Karaganda, Kazbek Bi District	24.04.24	21	49.74874 N, 73.1669 E
5	Karaganda, Kazbek Bi District	24.04.24	17	49.80769 N, 73.14454 E
6	Karaganda, Karaganda–Almaty Highway	24.04.24	15	49.74887 N, 73.167 E
7	Karaganda, Maikuduk Microdistrict	24.04.24	30	49.83559 N, 73.17974 E
8	Karaganda, Spassk Highway	24.04.24	5	49.75345 N, 73.16607 E
9	Karaganda Region, Prishakhtinsk Residential Area	25.04.24	2	49.92204 N, 73.06991 E
10	Karaganda, A. Bokeikhan District, E018	24.04.24	50	49.82488 N, 73.13184 E
11	Karaganda, A. Bokeikhan District, E018	24.04.24	35	49.83606 N, 73.11525 E
Zone II				
12	Karaganda Region, Nura District, Karkaraly Highway	22.04.24	20	50.66456 N, 71.46498 E
13	Karaganda Region, Nura District, Karkaraly Highway	22.04.24	14	50.61068 N, 71.46704 E
14	Karaganda Region, Nura District, Karkaraly Highway	22.04.24	36	50.3233 N, 71.52544 E
15	Karaganda Region, Nura District	22.04.24	5	50.25673 N, 71.56424 E
16	Karaganda Region, Nura District, Karatal Forest Plantation	23.04.24	2	50.30295 N, 71.61795 E

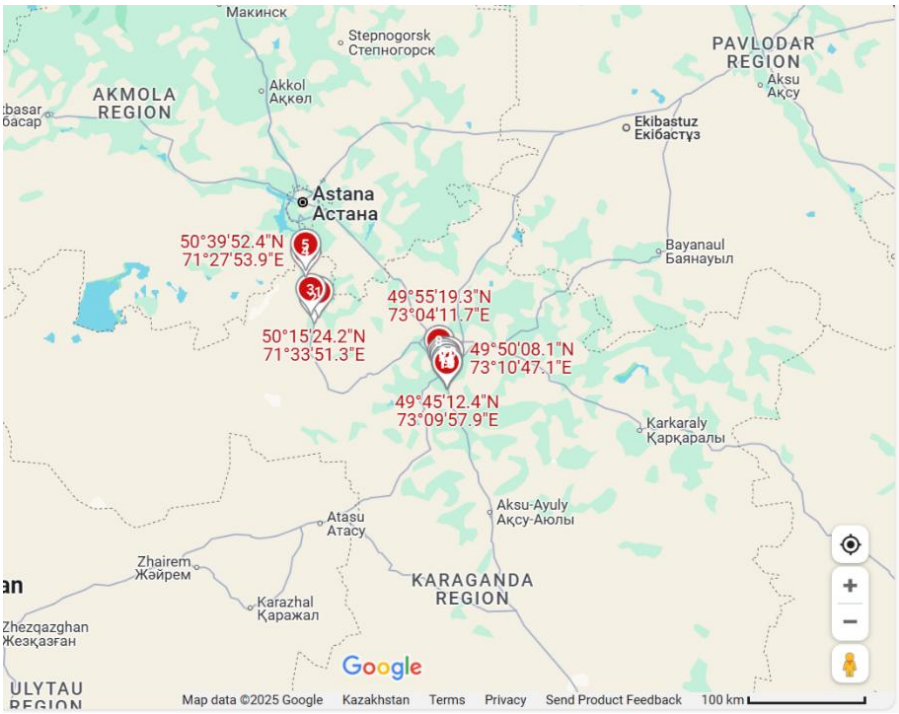

Figure 1. Geographic Distribution of Sampling Sites in the Karaganda Region

The initial mosquito generation develops in small water bodies formed by snowmelt. These shallow, sun-warmed pools provide a warm and favorable environment for larval development.

In Zone I, the water bodies were predominantly shallow and small puddles. In contrast, Zone II was characterized by water bodies with abundant green vegetation (Fig. 2).

a. b.

Figure 2. Water Bodies of the Karaganda Region a. In the vicinity of Karaganda city; b. Nura District

The division into zones was based on geographical, climatic, and precipitation factors to examine the variation in mosquito species distribution across the studied region.

The climate is sharply continental and arid, characterized by significant temperature fluctuations throughout the year and diurnally, as well as variability in weather conditions [12].

Maximum precipitation values (350–400 mm) were recorded in the Nura District, attributed to its location on the western slope of the low mountain range. The central part of the region exhibits a more uniform

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distribution of precipitation, averaging around 300 mm. The spring period accounts for approximately 25 % of the annual precipitation volume [13]. Several soil districts, zones, and subzones are distinguished within the region. The areas adjacent to the city of Karaganda belong to the subzone of Humic Kastanozems, while the Nura District is located within the kastanozems subzone.

Humic Kastanozems are characterized by a clayey, heavy and medium loamy granulometric composition, which slows down water infiltration into the ground and leads to water retention on the surface.

Kastanozems differ from Humic Kastanozems by having lower humus content, a tendency toward compaction, and the presence of readily soluble salts. These soils are formed under non-leaching water regimes typical of dry steppe and semi-desert environments, where surface water is rapidly absorbed into the soil [14].

To analyze species diversity, the Shannon index was used. This index allows for the comparison of species diversity across different communities. Pielou's evenness index (E), which is calculated based on the Shannon index, provides a measure of the uniformity of species distribution in terms of their abundance within a community [15].

To assess the similarity between the two zones, the Jaccard index was used. In the context of ecology and species diversity, this index allows for the evaluation of the degree of similarity in species composition between two different habitats [16].

To assess the similarity in species composition and structure between the two geographic zones, hierarchical cluster analysis was performed. The analysis was based on the relative abundance of g. *Aedes* mosquito larvae across all sampled habitats. We applied hierarchical agglomerative clustering using the complete-linkage method, in which inter-cluster distance is calculated as the maximum pairwise distance between observations belonging to different clusters. The analysis was performed using the Euclidean distance metric. The Bray-Curtis [17] dissimilarity index was used as the measure of distance, as it is appropriate for ecological data and accounts for differences in species abundances. The resulting dendrograms visually represent the degree of similarity between mosquito species in each zone, highlighting dominant species and indicating patterns of co-occurrence and species clustering.

Results

The samples contained representatives of the genera *Culex* and *Anopheles*, with *Aedes* larvae being predominant. In total, ten mosquito species belonging to the genus *Aedes* were identified: *Ae. (Ochlerotatus) caspius* Pallas, 1771; *Ae. (Och.) dorsalis* Meigen, 1830; *Ae. (Och.) flavescens* Müller, 1764; *Ae. (Rusticoidus) subdiversus* Martini, 1926; *Ae. (Och.) euedes* Howard, Dyar & Knab, 1913; *Ae. (Och.) cataphylla* Dyar, 1916; *Ae. (Och.) cyprius* Ludlow, 1920; *Ae. (Och.) annulipes* Meigen; *Ae. (Och.) cantans* Meigen, 1818; and *Ae. (Och.) communis* De Geer, 1776 (Table 2).

Table 2

Species Composition of *Aedes* Mosquitoes Collected in the Karaganda Region

Types/ Locations of samples	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Ae. (Och.) caspius Pallas, 1771	+	+	+		+	+	+		+	+	+				+	
Ae. (Och.) dorsalis Meigen, 1830	+	+			+	+	+			+	+				+	
Ae. (Och.) flavescens Muller, 1764				+		+		+								+
Ae. (Rus.) subdiversus Martini, 1926												+		+		
Ae. (Och.) euedes Howard, Dyar et Knab, 1913		+					+					+	+			
Ae. (Och.) cataphylla Dyar, 1916				+				+					+	+		
Ae. (Och.) cyprius Ludlow, 1920													+	+		
Ae. (Och.) annulipes Meigen,				+				+								

Continuation of Table 2

Types/ Locations of samples	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Ae. (Och.) cantans Meigen, 1818,				+						+	+					
Ae. (Och.) communis De Geer, 1776										+	+				+	
Note. The column number corresponds to the sample number from Table 1. Samples 1–11 belong to Zone I, and samples 12–16 belong to Zone II.																

The division into zones helped reveal differences in the distribution of mosquito species across the studied region.

It is worth noting that among the *Ae. subdiversus* specimens collected in the study area, individuals were found with a branched siphonal tuft 1-S (Fig. 3), whereas the species is described as having single siphonal tuft [9, 11]. In all other morphological characteristics, these individuals fully corresponded to the typical description of the species, leaving no doubt regarding the accuracy of their identification.

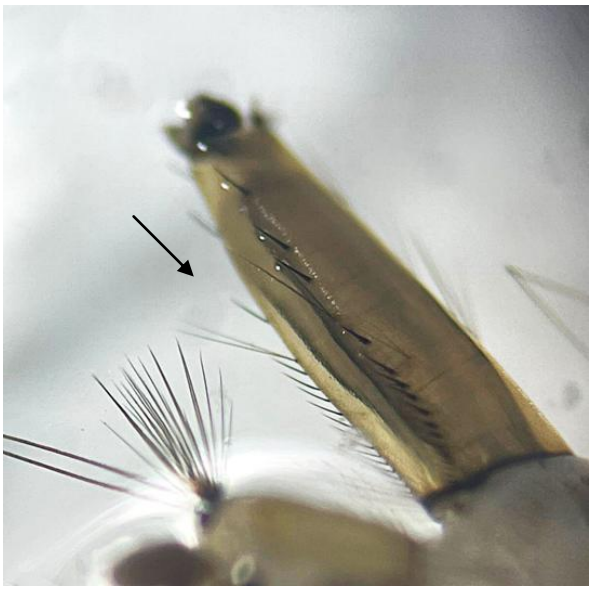

Figure 3**.** Siphonal tuft 1-S with two setae in *Ae. subdiversus*

The Shannon index (H) in the first geographic zone was 1.23, indicating relatively low species diversity within the mosquito community. Since the index value is below 1.5, this suggests that a few species are dominant.

The evenness (E) value of 0.59 indicates an uneven distribution of species abundance among the samples from Zone I (Table 1). A value below 0.7 also points to the dominance of several species.

According to the study, eight Aedes species were identified in the territories classified as Zone I, with a significant numerical dominance of *Ae. caspius and Ae. dorsalis* (Fig. 4).

The results for Zone II (Table 1) differ slightly. The Shannon index for this area was lower (H = 0.78) than in Zone I, indicating a lower level of species diversity. Seven Aedes species were recorded in this zone. The evenness value (E = 0.40) was also lower than in Zone I, suggesting a less even distribution of species abundance — i.e., a stronger dominance of a single species. In this region, *Ae. subdiversus* was the dominant species (Fig. 4).

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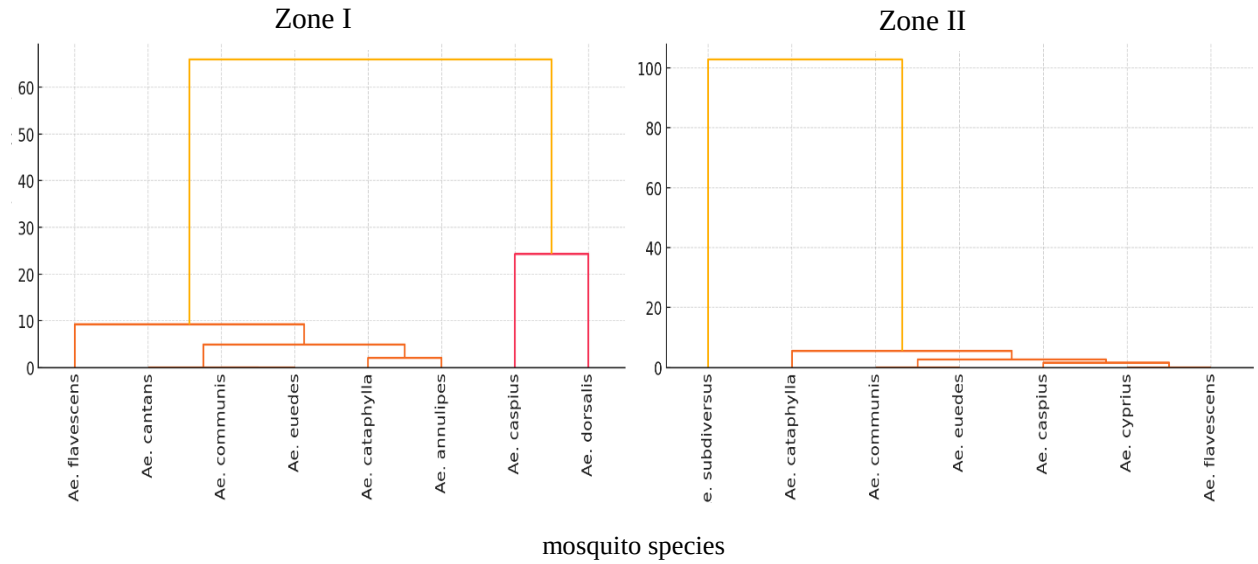

Figure 4. Cluster dendrograms based on the relative abundance of mosquito larvae

In both zones, a nearly equal number of g. *Aedes* species was recorded — eight in Zone I and seven in Zone II — but the species composition differed (Table 2). For instance, larvae of *Ae. cyprius*, *Ae. annulipes*, *Ae. cantans*, and *Ae. subdiversus* were not found in the water bodies of Zone I, whereas the latter species was dominant in Zone II. Conversely, *Ae. dorsalis* larvae were not found in Zone II, and *Ae. caspius* larvae were detected at very low abundance (3.9 %). In Zone I, however, these two species were predominant in terms of abundance. To show the similarities in the species composition of *Aedes* mosquitoes across two geographic zones, a cluster analysis was conducted based on the relative abundance of larvae. The dendrograms (Fig. 4) present the results of hierarchical clustering using the complete linkage method, where the distance between clusters is defined as the maximum distance between individual elements in each cluster. Euclidean distance was applied as the dissimilarity measure. The resulting dendrograms allowed for visualization of species associations based on their dominance within each zone. In Zone I, the most similar species in terms of abundance were *Ae. dorsalis* and *Ae. caspius*, forming a distinct cluster, which confirms their dominance in this area. The remaining species, which were less numerous, grouped into separate branches, indicating greater differences. In Zone II, *Ae. subdiversus* is clearly separated from the other species, reflecting its numerical dominance. The rest of the species form a compact cluster with minor differences in abundance (Fig. 4).

The Jaccard index was approximately 0.545, indicating a moderate level of similarity in mosquito species composition between Zone I and Zone II. About 54.5 % of the species recorded across both zones were found in both areas.

Discussion

We identified at least ten species of *Aedes* mosquitoes in the Karaganda Region, exhibiting heterogeneous patterns of distribution across the area. Notably, the presence of *Ae. annulipes*, *Ae. cantans*, *Ae. cataphylla*, *Ae. euedes*, *Ae. cyprius*, *Ae. dorsalis*, and *Ae. communis* has not been previously recorded in the studied territory.

Previously, the following *Aedes* species have been reported in the Karaganda Region: *Ae. (Rusticoidus) subdiversus* Martini, 1926; *Ae. (Ochlerotatus) rossicus* Dolbeskin, Gorickaja & Mitrofanova, 1930; *Ae. (Ochlerotatus) caspius* Pallas, 1771; *Ae. (Ochlerotatus) flavescens* Müller, 1764; *Ae. (Aedes) cinereus* Meigen, 1818; and *Ae. (Aedimorphus) vexans* Meigen, 1830 [10]. In our samples, larvae of *Ae. rossicus*, *Ae. cinereus*, and *Ae. vexans* were not detected. The spring generation of the first two species typically develops later than that of other g. *Aedes* species. *Ae. vexans* is also considered a thermophilic species [9, 10]. Since our sampling was conducted at the end of April, it can be assumed that the larvae of these species may appear in water bodies at a later time.

The Jaccard index indicated considerable similarity in species composition (six shared species), although each zone also supported distinct species not found elsewhere. In Zone II, a smaller number of water bodies was surveyed, which may lead to some adjustments in future studies. However, it is unlikely that the

species ratio between the zones will change significantly, as the numerical dominance of certain species is particularly pronounced. Zone I is characterized by moderate diversity, dominated by two species. A distinct cluster emerges comprising *Ae. dorsalis* and *Ae. caspius* (53.2 % and 28.9 %, respectively), the most abundantly represented species in the study area. Other species, including *Ae. communis, Ae. euedes,* and *Ae.* cantans, form separate clusters, indicating their low and similar encounter rates (Fig. 3).

Zone II exhibits extremely low diversity, with the absolute dominance of a single species. *Ae. subdiversus* stands out as a clear dominant with a substantial prevalence of 81.6 %, positioned distinctly apart from other species on the dendrogram. The remaining species are more evenly distributed, though their proportions are minimal (ranging from 1.3 % to 6.6 %) (Fig. 3).

The differences in species composition across the surveyed areas can primarily be attributed to ecological factors, such as the predominant types of water bodies, aquatic vegetation (Fig. 2), and soil types. These factors influence the mineral composition of the water and the persistence of temporary water bodies [16].

Conclusion

The species composition of blood-sucking mosquitoes of the genus *Aedes* was studied in the Nura District of the Karaganda Region, the city of Karaganda, and adjacent areas. The analysis revealed the presence of 10 g. *Aedes* species in the surveyed areas, with an uneven distribution. Dominant species were identified: in Zone I, *Ae. dorsalis* and *Ae. caspius* (53.2 % and 28.9 %, respectively), and in Zone II, *Ae. subdiversus* (81.6 %).

The identification of seven species (*Ae. annulipes, Ae. cantans, Ae. cataphylla, Ae. euedes, Ae. cyprius, Ae. dorsalis, Ae. communis*) not previously recorded in the Karaganda Region suggests a range expansion of *Aedes* mosquitoes, with important epidemiological implications for arbovirus transmission.

Additionally, the study identified a previously undescribed morphological variation in *Ae. subdiversus*, not reflected in existing identification keys.

The findings underscore the species diversity and the necessity for further studies on the fauna of bloodsucking mosquitoes, utilizing molecular methods, monitoring their abundance, and mapping their distribution in central Kazakhstan.

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Қарағанды облысының қансорғыш *Aedes (Diptera: Culicidae)* тұқымдасының масаларының түрлік құрамы

Жұмыста Қарағанды облысындағы (Қазақстан) масалардың түрлік құрамын зерттеу нәтижелері негізінде жасалған түрлердің тізбесі ұсынылған. Біздің нәтижелеріміз *Aedes* тұқымдасына жататын қансорғыш масалардың кем дегенде 10 түрінің бар екендігін көрсетті. Сонымен қатар, зерттеу атипті морфологиялық сипаттамаларды көрсететін үлгілерді анықтады, бұл жаңа вариация мүмкіндігін көрсетеді. Бұл нәтижелер осы морфологиялық тұрғыдан ерекшеленетін үлгілердің таксономиялық мәртебесін нақтылау үшін, әсіресе молекулалық әдістерді қолдана отырып, қосымша зерттеулер жүргізу қажеттілігін анықтайды. Сондай-ақ, жұмыста түрлердің әртүрлілігін және түрлердің популяциядағы көптігі бойынша біркелкі таралуының талдау нәтижелері келтірілген. Қарағанды қаласы мен оның маңайындағы *Aedes* туысының масаларының түрлік құрамы Қарағанды облысы Нұра ауданының масалар фаунасынан ерекшеленетіні көрсетілген. Облыс орталығының маңындағы ең көп кездесетін түрі *Ae. (Och.) dorsalis*, ал Нұра ауданында *Ae. (Rus.) subdiversus* түрі басым. Бұл зерттеу *Aedes* тұқымдасы масаларының биоәртүрлілігін және оның Орталық Қазақстандағы қоғамдық денсаулық сақтау үшін маңыздылығын түсінуге бағытталған болашақ жұмыстарға негіз қалайды.

Кілт сөздер: Aedes, Culicidae, жәндіктер, түр, Шеннон индексі, Пиелу индексі, Жаккар индесі, фауна.

Е.А. Шеруова, Ю.В. Андреева, У.Д. Андреева, А.К. Сибатаев

Видовой состав кровососущих комаров рода *Aedes* (*Diptera: Culicidae*) Карагандинской области

В настоящей работе представлен перечень видов, составленный на основе результатов изучения видового состава комаров в Карагандинской области (Казахстан). Наши результаты свидетельствуют о наличии не менее 10 видов кровососущих комаров рода *Aedes*. Кроме того, в ходе исследования были выявлены экземпляры, демонстрирующие атипичные морфологические характеристики, что указывает на возможность новых вариаций. Эти результаты подчеркивают необходимость дальнейших исследований, особенно с использованием молекулярных методов, для уточнения таксономического статуса этих морфологически отличных экземпляров. В работе также приведены результаты анализа видового разнообразия и равномерности распределения видов по их обилию в сообществе. Показано, что видовой состав комаров р. *Aedes* г. Караганды и его окрестностей отличается от фауны комаров Нуринского района Карагандинской области. Доминирующим по численности видом в окрестностях областного центра является *Ae. (Och.) dorsalis*, тогда как в Нуринском районе численно преобладает

Ae. (Rus.) subdiversus. Данное исследование закладывает основу для будущих работ, направленных на понимание биоразнообразия комаров рода *Aedes* и его значения для общественного здравоохранения в центральном Казахстане.

Ключевые слова: Aedes, Culicidae, насекомые, виды, индекс Шеннона, индекс Пиелу, индекс Жаккара, фауна.

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To the question of current species composition of blackflies (Diptera, Simuliidae) of the Irtysh river in Pavlodar region

The article presents a list of species diversity of the family Simuliidae identified in the Pavlodar Priirtyshye during the period from 2022 to 2024. In the basin of the middle reaches of the Irtysh River (from the border of the Maysky district to the Zhelezinsky district, and the border with Russia), the presence of 5 species of blackflies was noted. The identified blackflies belong to the Palaearctic and Holarctic species of plastic blackflies capable of living in polluted water bodies: *Wilhelmia equine (Linnaeus, 1758), Boophthora erythrocephala (De Geer, 1776), Odagmia ornata (Meigen, 1818), Argentisimulium noelleri (Friederichs, 1920), Simulium reptans (Linnaaeus, 1758).* The history of the study of blackflies in Kazakhstan and the studied area is presented. The frequency of occurrence and the biology of the species in the Pavlodar region are described. Dominant species are identified.

Keywords: blackflies, Simuliidae, species composition, habitat area, Irtysh.

Introduction

Over the past 10 years, active work has been carried out to reduce the participation of blood-sucking blackflies not only in the North-Eastern part of Kazakhstan, but also in many southern regions (Syrdariya River). The establishment and current species structure allow us to develop more effective methods of combating blackflies. In addition, knowledge of international species and changes in their dynamics allows us to control the level of pollution of water bodies.

The last inventory of species composition in the North-Eastern part of Kazakhstan took place about 20 years ago. During this period, it is customary to consider individual species in the relationship, as well as the emergence of new, more plastic to pollution species of blackflies.

The global blackfly fauna (family Simuliidae) currently includes 2,424 recognized species [1].

Members of this family are distributed across all continents, except the Arctic and Antarctic regions [2]. Many blackfly species are considered important bioindicators of freshwater quality and can be effectively used to monitor the ecological status of aquatic ecosystems. Beyond their ecological relevance, blackflies are of considerable interest due to their significant veterinary and public health importance. Certain species act as vectors of serious invasive and infectious diseases affecting both humans and domestic animals, including onchocerciasis, anaplasmosis, and tularemia [3].

In this context, comprehensive studies of the faunistic diversity and species composition of blackflies are essential across all regions of Kazakhstan. Accurate information on species distribution is a prerequisite for the implementation of effective vector control, preventive, and public health strategies. The present study aims to identify the species composition of blackflies inhabiting the Pavlodar Region along the Irtysh River and to provide a concise overview of the biometric characteristics of the species recorded.

Studies of blackflies in Kazakhstan have been carried out by Russian and Kazakh scientists in different years. The southern and eastern regions of Kazakhstan are considered the most researched [4]. In the 50s–80s, the researches are reflected in the works of Shakirzyanova M.S. [5], Rubtsov I.A. [6]. Zoogeographic species composition of midges of Kazakhstan was summarized in the work of Kenzhebaev Zh.K. [7], of Konurbayev E.O [8].

The Pavlodar region, through which the blackfly course of the Irtysh River flows, is not fully studied [9]. The first records on the species composition and population of blackflies in this area were made by Sinelshchikov V.A. [10]. Further study of blackflies was carried out in the context of finding measures to control nuisance gnats. The mass extirpation of gnats harmed the agricultural and economic activity of the region. In subsequent years, data on the study of the faunistic composition of the region were conducted by Gabdulin E.S. [11], Alshin A.R. [12]. On the territory of the Bayanaulsky mountain-forest range, the study of blackflies was also conducted by Alikhanov Sh.A. [13].

It is worth noting that the study of blackflies in the Pavlodar region has been conducted for many years as part of the annual fight against blood-sucking insects. The study includes studies of the fauna, distribution, dynamics, and density of blackflies, as well as their impact on the economic part of our region.

This article presents data on the study of the species composition of blackflies in the Pavlodar Irtysh region from May 2023 to September 2024.

Materials and methods

The studied material was collected during field research on the Irtysh River, from early May 2023 to September 2024 in the Pavlodar region using standard methods described by Rubtsov I.A. [14; 56]. 413 blackfly imagoes were studied and identified, including 91 males and 322 females. 82 larvae and more than 110 pupae were collected.

The larvae and pupae were collected from a substrate immersed in water: fallen tree branches, plastic debris floating in the water. After collection, the research material was placed in test tubes with 90 % ethanol at a temperature of ±20ºC. Total preparations were made using a binocular magnifying glass MBS-10 and a biological light microscope with a UCMOS03100KPA Altami camera, type BIO-1. Pictures of blackflies' habitat landscapes were taken with a Samsung Galaxy S23Ultra camera. Permanent preparations of the head, limbs, and genitals were made using the method of Rubtsov I.A. [14] and fixed in Euparal. Pictures were taken from temporary preparations, and then the preparations were disposed of.

The method of hatching imagoes from pupae was also used. Collection and hatching of imagoes were carried out according to the methods of Khalin A.V. [15]. For these purposes, the pupa was placed in a clean test tube with a moistened wet swab and sealed. Then, a few hours after hatching, the empty pupa (exuvium) and the midge imago were fixed with 90 % ethanol in the same test tube for further identification.

The studied material was identified to the species level by the modern system of the *Simuliidae* family [1]. Morphological diagnostics to the species level was carried out using the identifiers of Rubtsov A.I. [14], Yankovsky A.V. [18]. The main features in the diagnosis of imago forms were: the color of the legs, mouth apparatus, and genitals; in larvae, the color of the head capsule, the respiratory threads of the larvae, and the posterior attachment organ. Pupae were diagnosed by the following main features: the shape of the cocoon, the nature of the branching (weaving) of the cocoon.

The assessment of blackfly species biodiversity was carried out based on the Shannon index (H′), calculated using the formula:

$$H' = -\sum(p_i \times \log_2(p_i)),$$

where p_i is the proportion of individuals of species i relative to the total number of individuals in the district.

The evenness index (E) was calculated as:

$$E = H' / H'_{max}, \text{ где } H'_{max} = \log_2(S),$$

where S is the number of distinct species in the districts (with non-zero abundance). The calculation results and their interpretation are presented in the "Results and Discussion" section.

To ensure comprehensive coverage of the potential habitats of blackflies (Simuliidae), sampling sites were selected along the entire course of the Irtysh River within the Pavlodar region.

In each administrative district (Table), at least four sampling points were established, representing various types of watercourses (main river channel, floodplain areas, and river branches), flow velocities, and riparian biotopes characteristic of the region. The sampling points within each district were placed at approximately equal distances, with an average distance of about 100 km between districts.

Blackfly collection was carried out during peak periods of daily activity before noon and in the evening from 4:00 PM to 9:00 PM. Standardized methods were employed, including sweep netting, host-baited collection, and sampling of larvae and pupae from submerged substrates.

Species identification was based on the analysis of a combination of diagnostic morphological features. For accurate identification, larvae were collected, and adult flies (imago) were reared from mature pupae to enable species-level determination not only from the adult specimens but also based on the structure and branching pattern of the pupal cocoon filaments.

The number of samples and their spatial distribution adhered to established zoological standards for faunal surveys of the family Simuliidae [6, 14, 15, 16].

Potential biases are associated with the limited seasonal coverage of the sampling period. For example, non-bloodsucking species of blackflies may not have been captured using host-baited collection methods. For this reason, material was also collected directly from aquatic substrates. However, several authors have noted [6, 8] that in large and medium-sized rivers, predominantly bloodsucking blackfly species emerge. To increase sampling diversity, sweep-netting of riparian vegetation was also conducted.

The species Argentisimulium noelleri was represented in the collection by two female specimens. It was not detected in aquatic substrates; the two individuals were collected from vegetation. This may be because the emergence period for this species had already passed by the time of sampling, and the species is generally rare in the Pavlodar region. It is also noteworthy that no male specimens of this species were found. Males typically emerge 5–7 days earlier than females, which may explain their absence. Therefore, in the following season, we plan to begin sampling for this species 10–14 days earlier.

Sampling was not conducted during windy or rainy weather. The primary focus was on collecting from aquatic vegetation, which allowed for the identification of multiple developmental stages (larva, pupa, imago). This approach also enabled us to estimate the timing of emergence and assess the seasonal activity of blackfly species based on the developmental stage and maturity of larvae and pupae.

The permanent preparations made are stored in the collection of Orazbekova A.

Results and Discussion

As a result of the conducted study of the species composition of blackflies in the Pavlodar Irtysh region, the species composition of blackflies was clarified and supplemented, including 5 genera and 5 species of blackflies. The collection was carried out from the main channel of the Irtysh River.

Subfamily SIMULIINAE Newman, 1834

Tribe WILHELMIINI Baranov, 1926

Genus Wilhelmia Enderlein, 1921

Wilhelmia equine (Linnaeus, 1758)

The studied material. Imago: 162 females, 32 males collected. Date of collection: May, July 2023, June 2024.

Palearctic, European-Asian species.

A plastic, widespread European species, including in the European part of Russia.

In the Pavlodar region, during the period of mass flight, it was noted in the coastal river zone in various biotopes: forest-steppe, forest zones, and coastal shrub thickets. The larvae of this species actively populate coastal vegetation. An active bloodsucker, attacks humans and animals, the dominance index is 47 %.

Morphological identification was carried out based on the genitalia of both the female and male, as well as the respiratory filaments of the pupa. A distinctive feature of the female genital structure is the presence of spines: the bases of the genital plates are curved inward and bear spines. The anterolateral sclerotized areas of the genital fork are weakly developed (Fig. 1a). The pupa possesses eight respiratory filaments, two of which are adjacent to the pupal collar, forming a semicircle. In the central region, six smaller filaments are arranged laterally to the main filaments and directed anteriorly (Fig. 1b). In the male, a small apical spine is present at the base of the gonostylus. The gonostylus is approximately 4.5 times narrower than the gonocoxite and is slender. The base of the gonosternum is triangular and curved inward (Fig. 1c).

Subfamily SIMULIINAE Newman, 1834

Tribe NEVERMANNIINI Enderlein, 1921

Genus Boophthora Enderlein, 1921

Boophthora erythrocephala (De Geer, 1776)

The studied material. Imago: 28 females, 3 males collected. Date of collection: June 2023.

Palaearctic, European-Asian-North American species.

Widespread throughout most of Europe and Russia. It is also found in North Africa and the East Asian region. In the territory of the Pavlodar region in 2016, it was one of the dominant species according to collections [17]. In the 2023 collections, the species was not abundant, with a dominance index of 8 %; in 2024, it was not recorded in the collections.

Species identification was based on the morphology of the male and female genitalia, as well as the pupae. The pupa possesses six respiratory filaments. All filaments are nearly identical in diameter and size. Each group originates from a common base, splitting into three branches, with two filaments arranged dichotomously on each branch (Fig. 1d). The male genitalia exhibit distinctive characteristics — the

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gonostyles are short with a square-shaped base. On the inner surface of the gonostyle base, there are five small spines (Fig. 1e).

Subfamily SIMULIINAE Newman, 1834

Tribe SIMULIINI Newman, 1834

Genus Odagmia Enderlein, 1921

Odagmia ornata (Meigen, 1818)

The studied material. Imago: 12 females collected. Date of collection: June 2023.

Palaearctic, European-Asian-North American species.

This species of blackflies is a polyzonal and widespread European species. It also robs in the European part of Russia. According to scientists [18], it is distributed from the countries of northwestern Africa and Western Europe to Eastern Siberia and the Far East. The species is characteristic of forest-steppe landscapes. In the Pavlodar region, it is found in the coastal part of the Irtysh River and the adjoining forest-steppe zone. It attacked the feeder mainly in open biotopes. The species is not numerous in terms of dominance, 3 %.

Species identification was conducted based on the genitalia of females reared from pupae. The cocoon is simple, with an indistinct or absent pupal collar. The anterolateral angles are widely spaced, projecting laterally and dorsally. There are six respiratory filaments, originating as three main filaments, each of which branches dichotomously into two filaments (Fig. 1f).

Genus Argentisimulium Rubzov et Yankovsky, 1982

Argentisimulium noelleri (Friederichs, 1920)

The studied material. Imago: 2 females collected. Date of collection: May 2023.

Holarctic, European-Asian-North American species.

The habitat is mainly in most of the countries of foreign Europe and Russia. On the territory of the Pavlodar region, the species is very scarce. The presence of this species was not noted in the 2024 collections.

Species identification was based on the morphology of the female genitalia and legs. The stem of the furcal stalk is slender and approximately 1.5 times longer than the fork itself. The gonapophyses exhibit a deep, straight median incision. On the branches of the fork, the anterolateral projections are prominently developed and directed upward. The anal plates are rounded, and the cerci are also rounded and relatively small (Fig. 1g).

Genus Simulium Latreille, 1802

Simulium reptans (Linnaaeus, 1758)

The studied material. Imago: 118 females, 56 males collected. Date of collection: May 2023, August 2024.

Palearctic, European-Asian species.

On the territory of Kazakhstan, the presence of this species is noted for the first time. The species is widely distributed in most of Europe, including the European part of Russia. On the territory of Pavlodar region, it is an active bloodsucker with a high dominance index in the spring period — 42 %. It is found in the coastal part, it is mainly observed in open biotopes: under growths, tree and shrub thickets.

Morphological identification was carried out based on the genitalia of males (Fig. 1i) and females. A distinctive feature of the species is the structure of the pupal cocoon. Along the edge of the cocoon collar, there are large square fenestrae (windows) (Fig. 1h). Imago specimens were reared from mature pupae for morphological examination. In addition, molecular genetic analyses were conducted to confirm the species identification. The corresponding sequence data have been deposited in GenBank under accession numbers

PP396815 — https://www.ncbi.nlm.nih.gov/nuccore/PP396815.1,

PP396749 — https://www.ncbi.nlm.nih.gov/nuccore/PP396749.1,

PP400833 — https://www.ncbi.nlm.nih.gov/nuccore/PP400833.1,

PP400933 — https://www.ncbi.nlm.nih.gov/nuccore/PP400933.1,

PP400977 — https://www.ncbi.nlm.nih.gov/nuccore/PP400977.1.

Figure 1. Morphological features of black flies: (a) Female genitalia of Wilhelmia equine; (b) Pupal respiratory filaments of W. equine; (c) Male genitalia of W. equine; (d) Pupal respiratory filaments of Boophthora erythrocephala; (e) Male genitalia of B. erythrocephala; (f) Pupa of Odagmia ornate on substrate (plastic bag); (g) Female genitalia of Argentisimulium noelleri; (h) Cocoon collar of Simulium reptans; (i) Male genitalia of S. reptans

Based on the results of the conducted research, it can be concluded that the species diversity of gnats in the Pavlodar Irtysh region is quite poor. The Shannon diversity index in the Pavlodar Irtysh region was 1.042, with an evenness of 0.647. The total number of blackfly species was 5, and the average number of individuals was 82. Based on the species composition data for each district (Table), the index was calculated separately for each district (Fig. 2).

Table

Species composition of blackflies in the Pavlodar Irtysh region by districts (2023-2024)

Species name	Total imagos	Maysky District	Pavlodar City	Zhelezinsky District
Wilhelmia equine	194	101	19	74
Boophthora erythrocephala	31	18	0	13
Odagmia ornate	12	9	1	2
Argentisimulium noelleri	2	0	0	2
Simulium reptans	174	63	29	82

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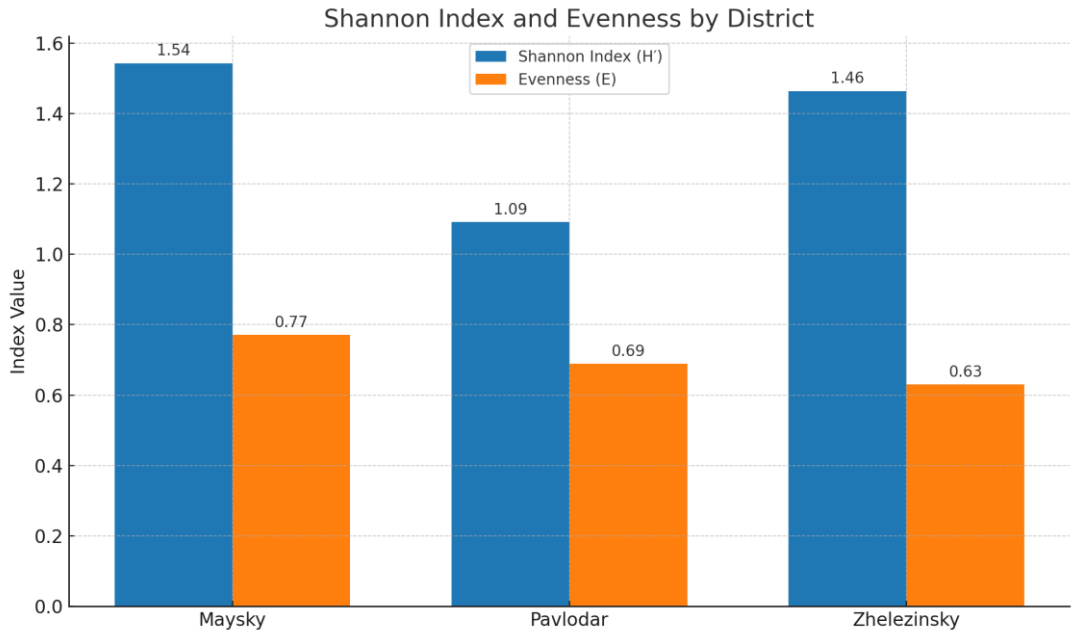

Figure 2. Shannon index and evenness values by district in the Pavlodar Irtysh region — from the border of Maysky District downstream to Zhelezinsky District (border with Russia) during the period from May 2023 to September 2024

As we can see from Table, among the five recorded species, the main dominant ones are *W. equine* and *S. reptans*. Figure 2 demonstrates that the blackfly community is generally balanced; however, despite this, the level of evenness remains approximately the same within each community (ranging from 0.6 to 0.7). The Shannon index increases both with the number of species and with greater evenness. However, the level of dominance (evenness) is not consistent. This indicates that in all three regions, one or more species (*W. equine* and *S. reptans*) dominate. In the Maysky District, species are distributed almost evenly (0.77); in the area near Pavlodar, there are dominant species, but not a complete skew (0.69); and in the Zhelezinsky District, clear dominance is observed along with a lower evenness value (0.63).

Such an uneven distribution indicates the dominance of certain taxa, which may suggest an ecological imbalance in the studied environment. However, many authors have noted that the low species diversity of blackflies is characteristic of lowland rivers [8], [6].

Active bloodsuckers attacking humans and animals in mid-May are midges — *W. equinum* and *S. reptans*. They are also absolute dominants. In the 2023 collections, blackflies of the species *Arg. noelleri* and *B. erythrocephala* were present in small quantities, but these species were not found in the 2024 collections. Although B. *erythrocephala* is a widespread eurybiont and synanthropic species that also develops in large rivers, withstanding significant temperature fluctuations, it is noticeable that its numbers in the Pavlodar region are declining.

In the Pavlodar Irtysh region, the widespread European species of blackflies, *S. reptans*, was identified for the first time. The presence of this species in Kazakhstan was noted for the first time. The data are confirmed by molecular genetic studies [19].

In the studies of Makatov T.K. [20], the fauna of the Pavlodar Irtysh region consisted of 6 genera and 9 species of midges. According to the data of 2016 [17], based on the results of our collections in 2023-2024, there are 5 species of blackflies in the fauna of the Pavlodar Irtysh region, belonging to 5 genera. A significant reduction in species diversity can be noted, and the presence today is of only more plastic species of blackflies. Since midges are amphibious animals, it can be assumed that less plastic species either disappeared from this range or make up a small percentage of the total number. Also, some abiotic factors could have had an impact: a decrease in oxygen in the water, high turbidity. It is not worth excluding the fact that, since 2015, every year, there has been carried out an active work in the region to reduce the number of midges. And there is a possibility that in the Pavlodar Irtysh region, species that are less flexible in processing will decrease in number over time.

Conclusion

The tribe *Simuliini* consists of genera widespread in the Northern Palearctic. In the fauna of the Pavlodar Irtysh region, the presence of 5 species of blackflies was noted. Including *S. reptans*, which is a new species for the fauna of Kazakhstan.

The most common species are *W. equinum* and *S. reptans*.

The plain and forest-steppe landscapes contribute to a relatively low level of blackfly species diversity, as well as the predominance of bloodsucking species. In the Pavlodar Irtysh region, the dominant species are those tolerant to water pollution. It is difficult to definitively attribute the reduction in blackfly species richness to specific anthropogenic factors, as large and medium-sized plain rivers are typically characterized by low species diversity. Moreover, rivers such as the Irtysh mainly support the emergence of hematophagous blackfly species, while non-bloodsucking blackflies are generally not dominant in such communities [6, 8, 14].

The species diversity of blackflies in the Pavlodar Irtysh region corresponds to its geo-hydrological regime. The lowest level of blackfly biodiversity was recorded in Pavlodar city; however, the evenness index here did not indicate strong dominance by individual species. A more balanced species distribution was observed in the Maysky District.

The results of the study on the blackfly fauna (Simuliidae) in the Irtysh River basin within the Pavlodar region may serve as a foundation for the development of an integrated ecological monitoring system for the region's aquatic ecosystems.

Large and medium-sized lowland rivers such as the Irtysh provide favorable conditions for the mass emergence of hematophagous blackfly species. The surveyed areas were dominated by Wilhelmia equina, Boophthora erythrocephala, and Simulium reptans species, characterized by a high degree of ecological plasticity. Their abundance can serve as an indicator of persistent pollution, eutrophication, and other forms of anthropogenic impact on aquatic environments. Therefore, the species composition of blackflies may be used as a sensitive indicator for assessing the ecological status of freshwater bodies.

Given these characteristics, it is recommended to implement regular monitoring of blackfly communities in strategically important sections of the Irtysh basin, particularly within populated areas, floodplain zones, and downstream of hydraulic structures. An optimal sampling frequency would be at least twice per season: once in the spring-summer period and once in the late summer-autumn period. This approach would allow for the detection of changes in species composition, early signs of ecological disturbances, and the timely development of measures for the management and protection of regional aquatic ecosystems.

In the future, monitoring activities are needed to track changes in species dynamics. The identification of a new species for the fauna of Kazakhstan confirms the necessity of continuing faunal surveys in the Irtysh region.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Orazbekova A.A**.: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing; **Akhmetov K.K.**: Formal analysis, Validation, Writing – review & editing; **Burkitbayeva U.D.**: Resources, Investigation, Project administration; **Aubakir A.S.**: Supervision, Writing – review & editing, Funding acquisition.

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Павлодар өңіріндегі Ертіс өзенінің шыбын-шіркейлерінің (Diptera, Simuliidae) қазіргі түрлік құрамы туралы мәселеге

Мақалада 2022-2024 жылдар аралығында Павлодар өңіріндегі Ертіс өзенінен тіркелген *Simuliidae* тұқымдасының түрлік әртүрлілігінің тізімі келтірілген. Ертіс өзенінің орта ағысы бассейнінде (Май ауданының шекарасынан Ресеймен шекаралас Железин ауданына дейін) шыбын-шіркейдің 5 түрінің бар екені анықталды. Анықталған шыбын-шіркейлер ластанған су айдындарында тіршілік ете алатын палеарктикалық және голарктикалық пластикалық түрлерге жатады, олар: *Wilhelmia equina* (*Linnaeus*, 1758), *Boophthora erythrocephala* (*De Geer*, 1776), *Odagmia ornata* (*Meigen*, 1818), *Argentisimulium noelleri* (*Friederichs*, 1920), *Simulium reptans* (*Linnaeus*, 1758). Қазақстандағы және зерттелген аумақтағы шыбын-шіркейлерді зерттеу тарихы қарастырылған. Түрлердің таралу жиілігі көрсетіліп, Павлодар өңіріндегі олардың биономикасы сипатталған. Басым түрлер анықталған.

Кілт сөздер: шыбын-шіркейлер, *Simuliidae*, түрлік құрамы, таралу аймағы, Ертіс.

А.А. Оразбекова, К.К. Ахметов, У.Д. Буркитбаева, А.С. Әубәкір

К вопросу о современном видовом составе мошек (Diptera, Simuliidae) на реке Иртыш в Павлодарской области

В статье приведен перечень видового разнообразия семейства Simuliidae, выявленного в Павлодарском Прииртышье в период с 2022 по 2024 год. В бассейне среднего течения реки Иртыш (от границы Майского района до Железинского района и границы с Россией) отмечено присутствие 5 видов мошек. Выявленные мошки принадлежат к палеарктическим и голактическим видам пластиковых черных мух, способных обитать в загрязненных водоемах: *Wilhelmia equine (Linnaeus, 1758), Boophthora erythrocephala (De Geer, 1776), Odagmia ornata (Meigen, 1818), Argentisimulium noelleri (Friederichs, 1920), Simulium reptans (Linnaaeus, 1758).* Приведена история изучения черных мух в Казахстане и на изучаемой территории. Указана частота встречаемости и описана биономика видов для Павлодарской области. Определены доминирующие виды.

Ключевые слова: мошки, Simuliidae, видовой состав, ареал обитания, Иртыш.

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Peculiarities of the root-suckering ability of *Hippophae rhamnoides* L. *plants* (East Kazakhstan region)

The article presents data on the root-suckering ability of *Hippophae rhamnoides* L. under various ecological and geographical conditions: in natural populations, at a breeding site, and in the introduction population of the Altai Botanical Garden. Based on age structure, the natural populations of the species are classified as fast-growing and stable. The lifespan of plants ranges from 16 years in the Karatal population to 32 years in the Tersayryk population. In culture, some forms and seedlings are 38 years old. By the number of root shoots *H. rhamnoides* L. in natural habitats and in the introduced population (clumps) the proportion of plants of the first and second age groups is high. In natural populations there are from 540 pcs/ha to 5173 pcs/ha of plants of the first age group, in the clumps of the introduced population from 1353 pcs/ha to 2076 pcs/ha, which in percentage terms is 30–79 %, 50–69 %, respectively. In the second age group in natural habitats there are plants from 380 pcs/ha to 556 pcs/ha, in clumps — from 457 pcs/ha to 844 pcs/ha. Undoubtedly, the influence of genes and environmental factors on the high root-suckering ability of plants is affected. The most optimal number of root suckers from 16 to 30 pcs per 12 m^2 in a breeding garden allows obtaining high-quality planting material. *Hippophae rhamnoides* L. of the East Kazakhstan ecotype is characterized by high root-suckering ability, winter hardiness, and longevity, which makes it suitable for use as a reclamation plant.

Keywords: Hippophae rhamnoides L., species, root shoots, clump, population, selection plot, seedling, phytocenosis.

Introduction

Hippophae rhamnoides L. (Sea buckthorn) is a Eurasian species that belongs to the family Elaeagnaceae Juss., has attracted attention since ancient times as a medicinal, food, vitamin, ornamental and soil-fortifying plant. I.P. Eliseev [1, 2] subdivides *H. rhamnoides*, growing in the territory of the CIS, into several climatypes: Siberian, Central Asian, Caucasian and Baltic. The Siberian climatype unites all populations of Transbaikalia, the vast Sayan-Altai Mountain region and the East Kazakhstan region. This climatype is characterized by a shorter vegetation period and outstanding frost resistance.

H. rhamnoides, a large shrub 0.7–high 3,5 м, less often a tree up to high 10 m with a well-developed superficial root system. The roots are bare, thick, long, have a loose anatomical structure, the mechanical tissue in them is poorly developed, they are relatively easy to break, have the ability to give abundant root shoots. They have numerous nodules with nitrogen-fixing bacteria. The ability of sea buckthorn to produce root shoots is one of its adaptive capabilities.

The impact of man, which is taking on an ever-widening scale on nature, with irrational and short-sighted management of its gifts can lead to innumerable troubles and global catastrophes. Open-pit mining of minerals disrupts tens of thousands of hectares of valuable lands, which must be subject to mandatory restoration [3, 4, 5, 6, 7]. Reclamation and rehabilitation of natural landscapes disturbed by man are important socio-economic and scientific-technical problems. Research on forest reclamation is broad in scope, comprehensive and practical in nature.

Sea buckthorn plays a significant ecological role, performing soil-protective and water-protective functions. The high root-suckering capacity of sea buckthorn is used to fix sand, dunes, ravines, gullies, railway slopes, landslide slopes and scree in mountainous areas, coastal mountain rivers, protect roads from snowdrifts, etc. It is often used as an ameliorant in near and far abroad countries: Russia, Kyrgyzstan, Georgia, the Czech Republic, Slovakia, China [8, 9, 10, 11]. In Kazakhstan, this species is considered a promising crop for the reclamation of industrial waste dumps of the Sokolovsky iron ore quarry [12] and at mining enterprises in Northern Kazakhstan [13]. Its use in forest formation process is promising in many regions of Kazakhstan, as it has the potential to grow in extreme conditions, including on the Mangyshlak Peninsula, according to the Mangyshlak Experimental Botanical Garden of Aktau, it grows well in an extra-arid climate, on gray-brown soils that are saline everywhere. The relevance of this study lies in assessing the root-suckering ability

of natural populations of *H. rhamnoides* in the East Kazakhstan region for further use in land reclamation and restoration.

The main objective of the study is to study natural populations of *H. rhamnoides* in the East Kazakhstan region and under introduction conditions with identification of the characteristics of root-suckering ability.

Materials and methods

Work on the study of the root-suckering ability of H. rhamnoides were carried out in natural populations of the East Kazakhstan region and in the conditions of the Altai Botanical Garden (ABG) during the introduction of clonal and seed material. Work in the Kenderlyk, Shetlasty, Tersayryk, Topkain, Kaindysu and Karatal populations was carried out using the route method. The names of the populations are given in accordance with the geographical names of their places of growth by the name of the rivers. Considering the clump-shaped arrangement of sea buckthorn in natural populations, test plots were laid out in all populations according to a single principle.

The study of the age composition of sea buckthorn and its abundance was carried out on sites, the number and area of which were laid out depending on the share of the species in various phytocenoses. The size of the trial plots was from 0.16 to 0.55 ha (120×40 m), (70×70 m), (65×85 m), (50×100 m) (40×40 m), (30×60 m), (25×40 m), depending on the number of individuals, usually this number reached from 92 to 373 specimens, the configuration was mosaic.

The unit of measurement of age class in natural sea buckthorn populations, taking into account the beginning of fruiting and life expectancy, was adopted as five years. All plants were distributed into five age groups according to the methods developed by the M.A. Lisavenko Siberian Research Institute of Horticulture [14].

When determining the maximum lifespan of plants in nature, plants with the greatest height and trunk diameter were cut down, the cuts were processed with sandpaper, and then the annual rings were counted. In introduction experiments, the lifespan of forms brought from nature by root shoots and seedlings obtained from free pollination was recorded based on the fact of plant death. In culture, in clumps, the age composition was studied using the above-mentioned method.

When laying out a breeding plot in 2008 in the Altai Botanical Garden in Ridder, the following planting scheme was used for seedlings from free pollination of local reproduction at the age of three and four years: 4 m between rows and 2 m in a row, with a placement of 1250 pcs/ha. The plot is located on the southeastern slope of Belkina. The soil where sea buckthorn grows is well tied to the relief and is represented by chernozem-like dusty loams (humus content of 6.45 %), quite rich in nitrogen (127 kg/ha), but has some deficiency in phosphorus. The validity and reliability of the data is ensured by a significant volume of research conducted in natural populations, in culture and on the breeding plot.

Results and discussion

Natural thickets of *H. rhamnoides* in the Republic of Kazakhstan in the 80s of the last century occupied 2640 hectares, with their discontinuous (disjunctive) range they enter the East Kazakhstan region and occupy 680 hectares [15]. Within the range, the species was studied in six populations in the river valleys: Tersayryk, Shetlasty, Kenderlyk, Kaindysu, Topkain and in inter-dune depressions in the Karatal sands (Fig.) [16, 17]. Edaphic factors in the valleys of mountain rivers in almost all geographical zones of sea buckthorn growth are similar — these are sandy and pebble shoals of rivers with the inclusion of alluvial silt deposits. In geographically isolated populations of mountain river valleys, the moisture conditions are also similar.

Figure. Natural populations of *Hippophae rhamnoides* L.

In the morphogenetic process of this species, geographical isolation is of primary importance. Eliseev emphasizes that the floodplain as an ecological niche is secondary for sea buckthorn, since the valleys of mountain rivers have a relatively young geological age, and their floristic composition is always formed due to the surrounding zonal vegetation [2].

Phytocenoses of populations consist, depending on the altitude above sea level — from 650 m (Karatal) to 1200 m (Tersayryk) of sea buckthorn, woody deciduous, shrubby and herbaceous plants of the local floodplain flora. The tree layer of the studied populations is poorly developed. Single species include: *Populus laurifolia* Ledeb., *P. pilosa* Rehd., *P. alba* L., *Betula tianschanica* Rupr., *Salix tenuijulis* Ledeb., *S. viminalis* L., *S. caspica* Pall., *Crataegus altaica* Lange. In the second tier it is accompanied by: *Lonicera tatarica* L., *Viburnum opulus* L. In the shrub layer in great abundance — *Berberis heteropoda* L. Less common is *Myricaria dahurica* (Willd.) Ehrenb., *Rosa laxa* Retz. The grass cover is represented by species of steppe and semi-desert vegetation: *Artemisia sericea* Weber ex Stechm., *Calamagrostis epigeios* Steud., *Phragmites australis* (Cav.) Trin. ex Steud., *Sophora alopecuroides* L., *Paeonia anomala* L., *Glycyrrhiza aspera* Pall. These plants can serve as a basis for the creation of artificial phytocenoses in the steppe and semi-desert zones of the Republic of Kazakhstan. With the melioration and reclamation of lands, it is possible to organize phytocenoses of sea buckthorn similarly to natural phytocenoses.

When studying the number and age composition of the populations, five groups were identified: young, mid-season, maturing, ripe and overmature. In 7 the trial plots, a large number of young plants under 5 years of age are noted, in percentage terms, they make up an average of 62 %. The largest number of root shoots, young plants 5173 pcs/ha are in the Shetlastinskaya population, which is 79.8 % (Table 1).

Table 1

The number of plants of *H. rhamnoides* by age categories in natural populations

Population	Age category	Number of plants per 1 piece/ha	% of total
Kendyrlyk	young animals (1–5 years)	1740	65.0
	middle-aged (6–10) years)	482	18.0
	maturing (10–15 years)	148	5.5
	ripe (16–20 years)	106	4.1
	overmature (21–25 years)	197	7.4
total		2673	100
Kaindysu	young animals (1–5 years)	540	30.7
	middle-aged (6–10 years)	415	23.6
	maturing (10–15 years)	605	34.4
	ripe (16–20 years)	135	7.7
	overmature (21–25 years)	65	3.6
total		1760	100

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Continuation of Table 1

Population	Age category	Number of plants per 1 piece/ha	% of total
Tersayryk	young animals (1–5 years)	2360	72.6
	middle-aged (6–10 years)	380	11.7
	maturing (10–15 years)	368	11.3
	ripe (16–20 years)	86	2.6
	overmature (21–25 years)	53	1.8
total		3247	100
Shetlasty	young animals (1–5 years)	5173	79.8
	middle-aged (6–10 years)	556	8.6
	maturing (10–15 years)	483	7.5
	ripe (16–20 years)	123	1.9
	overmature (21–25 years)	143	2,2
total		6478	100
Karatal	young animals (1–5 years)	367	22.0
	middle-aged (6–10 years)	272	16.3
	maturing (10–15 years)	162	9.7
	ripe (16–20 years)	869	52.0
total		1670	100
Topkain	young animals (1–5 years)	216	16.7
	middle-aged (6–10 years)	197	15.3
	maturing (10–15 years)	284	22.0
	ripe (16–20 years)	319	24.8
	overmature (21–25 years)	274	21.2
total		1290	100

There are two times less of them in the Tersayryk population 2360 pcs/ha (72.6 %), three times less in the Kenderlyk population — 1740 pcs/ha (65.0 %) and 9.5 times less in the Kaindysu population — 540 pcs/ha (30.7 %). In the latter population, the number of root shoots is affected by anthropogenic impact and proximity to the settlement of Akzhar village. The local population collects firewood and grazes cattle. The data in Table 1 indicate high percentages of plants in the first group (young plants) in relation to the total number. This group occupies a leading position in each of the populations, from 30.7 % in the Kaindysu population to 79.8 % in the Shetlasty population. The reasons for such differences are various ecological and geographical conditions, as well as numerous influences of biotic and abiotic environment of populations, which to varying degrees affect the factors determining their age structure. Thus, our studies confirm that sea buckthorn plants of natural populations have a high root sucker capacity. Reproduction of sea buckthorn in natural populations mainly occurs due to the formation of root suckers, which are associated with the mother plant throughout their life and form clumps. For example, in the Kenderlyk population there are areas where there are 9–18 root suckers per 1 m^2, which theoretically reaches a number of 90 thousand pcs/ha. The clumps are usually unisexual, sometimes mixed with a predominance of one or another sex. The habitat of sea buckthorn plays a certain role in the ability to produce root suckers. As studies have shown, in those populations where a greater number of plants of the first group are noted, young plants aged from 1 to 5 years are observed, basic, similar complexes of physical-geographical and biocenotic conditions. These are the Shetlasty, Kenderlyk, and Tersayryk populations. They have the principle of ecological compliance according to abiotic conditions: flat surface, hydrographic factor, river valleys: Shetlasty, Kenderlyk, Tersayryk and soil rubble-clayey with the same fertility necessary for the growth and development of plants, as well as the formation of root shoots. The hydrogeological regime of rivers during flooding in early spring contributes to an increase in soil fertility, the content of water and mineral nutrients in the soil. In the Karatal population, only 20 % of the total number of plants are young plants (root shoots), this is explained by the special habitat of sandy dry soils. The lifespan of plants depends on the habitat and varies from 16 years in

the Karatal population to 32 years in the Tersayryk population. In the Shetlasty and Kenderlyk populations, lifespan reaches 22–26 years.

There is little seed-based undergrowth, since seeds germinate poorly in sodded areas. Adult plants grown from seeds are confined mainly to the river bed. About the good root-suckering ability of sea buckthorn This is indicated by the fact that more than half of the forms isolated according to economically valuable characteristics had root suckers.

In the 80s and 90s of the last century, 68 best forms of sea buckthorn from five populations were brought to the Altai Botanical Garden, which were cloned by separating root shoots of 4–8 pieces from each mother plant. This method allowed it to be introduced into culture faster, since their survival rate was high 60–70 %, despite the fact that some forms had to be transported from one place to another for 1-2 days, up to 25 days in total. The plants, which were in bags, were constantly moistened by immersing them in water. During the introduction, all of them showed good and extreme adaptive plasticity. The same plants that did not have root shoots were brought from the same populations by seeds, from 52 mother individuals, most of them were preserved and served as the source material for selection, seedlings of three generations were obtained from them.

Evidence of the high adaptability of *H. rhamnoides* in the Altai Botanical Garden is also the formation of three clumps with a total area of 10,575 m^2 from 335 to 580 m^2 due to root shoots from male and female individuals, actively occupying living space.

Currently, intensive vegetative propagation is observed both inside the clumps and along the edges. Their density and size have been maintained at almost the same level for 35 years, since 1983. Their rejuvenation is observed due to an increase in the proportion of individuals of the first and second age groups. Abundantly fruiting plants aged 15–28 years grow in these clumps. Each clump contains plants represented by five age groups. The nature of the preservation of age spectra over a long period of time indicates the ability of the species to exist in culture. The number of plants per hectare is approximately within the same range as in nature, from 3,009 pcs in clump № 1 to 3,890 pcs in clump № 2 (Table 2).

Table 2

***H. rhamnoides* plants by age categories in the clumps of the Altai Botanical Garden**

Clump of trees	Age category	Number of plants per piece/ha	% of total
Clump № 1	young animals (1–5 years)	2076	69.0
	middle-aged (6–10 years)	457	15.2
	maturing (10–15 years)	241	8.0
	ripe (16–20 years)	120	4.0
	overmature (21–25 years)	115	3.7
total		3009	100
Clump № 2	young animals (1–5 years)	1353	50.2
	middle-aged (6–10 years)	844	21.7
	maturing (10–15 years)	443	11.4
	ripe (16–20 years)	265	6.8
	overmature (21–25 years)	385	9.9
total		3890	100
Clump № 3	young animals (1–5 years)	1993	57.2
	middle-aged (6–10 years)	651	18.6
	maturing (10–15 years)	374	10.8
	ripe (16–20 years)	190	5.5
	overmature (21–25 years)	276	7.9
total		3483	100

The largest number of root suckers, young plants 2076 pcs/ha in clump № 1, which is 69.0 %. Less than 57.2 % in clump № 3 and 50.2 % in clump № 2. In clump № 1, there is currently a keen sense of competition in conquering the area, since the number of root suckers included in the first group is 69 %. A study of the

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age composition in clumps growing in the garden allows us to conclude that due to the high percentage of plants in the first and second age groups, they belong to the category of fast-growing.

An important property of sea buckthorn in the East Kazakhstan region is winter hardiness. Thus, plants growing in the Altai Botanical Garden in a sharply continental climate with cold, long winters, often reaching a critical mark of 40–42ºC and repeated thaws with temperature drops of 18–20ºC, withstand critical temperatures without damage.

Testing of sea buckthorn plants from the Kenderlyk forestry enterprise (East Kazakhstan region) in the Novgorod region (Russia) by V.A. Fefelov [18] allowed us to conclude that it has outstanding frost resistance, which is genetically determined.

In the first two years after planting 96 seedlings on the selection plot in 2018, a large number of root suckers were formed: from 2 to 147 pcs per each plant. Apparently, the age of the seedlings had a positive effect on the root sucker capacity. This is approximately 10,500 pcs/ha ready for planting, the same number per hectare was noted during reclamation in the Volgograd region (Russia) [19]. They were divided into four groups: with a small number from 2 to 36 pcs per seedling, which is 57.6 %; with an average from 37 to 73 pcs — 21.7 %; large from 74 to 110 pcs — 15.2 % and very large from 111 to 147 pcs — 5.5 % (Table 3).

Table 3

Distribution of root suckers *H. rhamnoides* by groups of some indicators (by quantity and in %) on the selection site

Feature	Distribution of features by groups	Quantity, pcs	% ratio
Number of root shoots	few (2–36 pcs)	53	57.6
	average (37–73 pcs)	20	21.7
	many (74–110 pcs)	14	15.2
	very many (111–147 pcs)	5	5.5
Number of root shoots ready for planting	few (1–15 pcs)	58	62.4
	average (16–30 pcs)	23	24.7
	many (31–47 pcs)	12	12.9
Root shoots ready for planting to the total number	average (21.9–45.0)	55	67.9
	high (45.1–75.0)	26	32.1
Root suckering ability of female plants	few (2–36 pcs)	14	38.8
	average (37–73 pcs)	8	22.2
	many (74–110 pcs)	6	16.6
	very many (111–147 pcs)	8	22.2
Root suckering ability of male plants	few (2–36 pcs)	8	32.0
	medium (37–73 pcs)	4	16.0
	many (74–110 pcs)	10	40.0
	very many (111–147 pcs)	3	12.0
Height of root shoots	tall (174–260 cm)	37	38.9
	medium (83–73 cm)	58	61.1
Max. height of root shoots	low (10–50 cm)	36	37.5
	medium (51–91 cm)	49	51.0
	tall (92–120 cm)	11	11.5

When calculating the number of root suckers ready for planting, the obtained data are divided into three groups: few root suckers from 1 to 15 pcs. have 62.4 % of plants; average number from 16 to 30 pcs have 24.7 % of plants; many from 31 to 47 pcs — 12.9 %. The most optimal number of root suckers 24.7 % is in the second group. The fewer root suckers from the maternal and paternal individuals, the more plants are ready for planting with an optimal height of 51 to 91 cm. The accounting of root shoots during the establishment of the garden in the Altai Botanical Garden with seedlings, the origin of which is from different populations, showed that this ability is preserved from ecotypic differences. More root shoots were given by

seedlings from the Shetlasty and Kenderlyk populations, less from the Karatal. The introduction experiment on sea buckthorn in the Altai Botanical Garden showed that the varieties brought from the Research Institute of Plant Breeding named after M.A. Lisavenko practically do not produce root shoots, do not have such ability. This allows us to draw a conclusion about the influence of genetically diverse forms on such a biological property as plant reproduction. In the first years, sea buckthorn is characterized by higher growth energy. If we take into account the formation of root suckers by female and male plants, a higher percentage of output from 74 to 110 pcs is observed in male plants in the third group 40.0 % compared to 16.6 % in females. In the remaining groups: few, average and very many — the data in percentage terms are comparable. The percentage with a small number of shoots is almost the same, 38.8 % was obtained from female and 32.0 % from male, with an average number of 22.2 % for female and 16.0 % for male. In the group — very many root shoots, a predominance of 22.2 % was noted in female plants.

Conclusion

In this work, the root-suckering ability of sea buckthorn and the nature of its natural distribution in various ecological and geographical conditions and in culture in the East Kazakhstan region are determined. The root-suckering ability of sea buckthorn is one of its adaptive capabilities. The lifespan of plants in nature and in culture is also important, for which it reaches 22–35 years. Data from the experience of introducing varieties of sea buckthorn bred by the M.A. Lisavenko Research Institute of Horticulture of Siberia in the Altai Botanical Garden showed that their varieties are short-lived (16–20) years, and almost do not form root suckers at any age. *H. rhamnoides*, growing in the East Kazakhstan region, has a high root-suckering capacity, which allows us to recommend it for the restoration of lands, slopes, ravines, etc.

Sea buckthorn plants from natural populations have a high root-suckering capacity, which is confirmed by our studies in natural populations, in culture and on a selection site in the conditions of Eastern Kazakhstan. The degree of influence of genes and environmental factors on the high root-suckering capacity of plants is unambiguous. In terms of the number of plants per 1 ha in nature, the data range from 2673 pcs/ha in the Kenderlyk population to 6478 pcs/ha in the Shetlastinskaya population and 10500 pcs/ha in the breeding plot in the Altai Botanical Garden. In order to increase the economic efficiency of reclamation processes, it is advisable to use sea buckthorn plants from Eastern Kazakhstan for their high root-suckering ability, longevity and winter hardiness.

We recommend using 3-4 year old plants for planting, deepening them 8 cm from the root collar, and using forms and seedlings taken from the Kenderlyk, Shetlasty, and Tersayryk populations in steppe regions, and the Karatal populations on sandy soils.

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Author Contributions

The manuscript was written with the participation of all authors. All authors approved the final version of the manuscript. **Vdovina T.A.** – Research in natural populations, Methodology; **Lagus O.A.** – Research in the breeding garden, Editing; **Isakova E.A.** – Data curation, Research in the breeding garden.

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Hippophae rhamnoides L. өсімдігінің тамыр атпаларын түзу қабілетінің ерекшеліктері (Шығыс Қазақстан облысы)

Мақалада *Hippophae rhamnoides* L. өсімдігінің әр түрлі экологиялық-географиялық жағдайларда: табиғи популяцияларда, селекциялық учаскеде және Алтай ботаникалық бағының интродукциялық популяциясында тамыр атпаларын түзу қабілеті жөніндегі деректер келтірілген. Жас құрылымына қарай табиғи популяциялар жылдам өсетін және тұрақты топқа жатқызылды. Өсімдіктердің тіршілік ұзақтығы қаратал популяциясында 16 жылды, терісайрық популяциясында 32 жылды құрайды. Дақылдық жағдайда кейбір түрлер мен көшеттер 38 жылға дейін өмір сүреді. Табиғи мекендерінде және интродукциялық популяциядағы *Hippophae rhamnoides* L. өсімдіктерінде тамыр атпаларының саны бойынша бірінші және екінші жас топтарының үлесі жоғары. Табиғи популяцияларда бірінші жас тобына жататын өсімдіктер саны 540 данадан 5173 дана/га-ға дейін, ал интродукциялық популяциядағы шоғырда 1353 данадан 2076 дана/га-ға дейін жетеді, бұл 30-79 % және 50-69 % аралығында. Екінші жас тобындағы өсімдіктер саны табиғи мекендерде 380-556 дана/га, ал интродукциялық популяцияда 457-844 дана/га аралығында өзгереді. Өсімдіктің жоғары тамыр атпаларын түзу қабілетіне генетикалық факторлар мен сыртқы орта жағдайларының әсері айқын байқалады. Селекциялық бақта 12 м²-ге шаққанда 16-30 тамыр атпасының болуы сапалы егу материалын алуға мүмкіндік береді. Шығыс Қазақстан экотипіндегі *Hippophae rhamnoides* L. өсімдігі жоғары тамыр атпаларын түзу қабілетіне ие болғандықтан, оны мелиорациялық өсімдік ретінде қолдану ұсынылады. Бұл түр жоғары қысқа төзімділігімен және ұзақ өмір сүруімен ерекшеленеді.

Кілт сөздер: *Hippophae rhamnoides* L., түр, тамыр атпалары, өсімдік шоғыры, популяция, селекциялық учаске, көшет, фитоценоз.

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Особенности корнеотпрысковой способности растений *Hippophae rhamnoides* L. (Восточно-казахстанская область)

В статье представлены данные по корнеотпрысковой способности *Hippophae rhamnoides* L. в различных эколого-географических условиях: в природных популяциях, на селекционном участке и в интродукционной популяции Алтайского ботанического сада. По возрастной структуре природные популяции вида отнесены к категории быстрорастущих и устойчивых. Продолжительность жизни растений варьирует от 16 лет в каратальской популяции до 32 лет в терсайрыкской. В культуре у некоторых форм и сеянцев возраст равен 38 годам. По количеству корнеотпрысков Hippophae rhamnoides L. в естественных местообитаниях и в интродукционной популяции (куртинах) высока доля растений первой и второй возрастных групп. В природных популяциях насчитывается от 540 шт/га до 5173 шт/га растений первой возрастной группы, а в куртинах интродукционной популяции от 1353 шт/га до 2076 шт/га, что в процентном выражении составляет 30–79 % и 50–69 %, соответственно. Во второй возрастной группе в естественных местообитаниях произрастает растений от 380 шт/га до 556 шт/га, в куртинах от 457 шт/га до 844 шт/га. Несомненно сказывается влияние генов и факторов внешней среды на высокую корнеотпрысковую способность растений. Самое оптимальное количество корнеотпрысков от 16 до 30 шт. на 12 м^2 в селекционном саду позволяет получать качественный посадочный материал. *Hippophae rhamnoides* L. восточно-казахстанского экотипа характеризуется высокой корнеотпрысковой способностью, зимостойкостью и долголетием, что позволяет рекомендовать его в качестве мелиоративного растения.

Ключевые слова: Hippophae rhamnoides L., вид, корнеотпрыски, куртина, популяция, селекционный участок, сеянец, фитоценоз.

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Field Evaluation and Diversity of 238 Global Chickpea (*Cicer arietinum* L.) Genotypes Grown in South-East Kazakhstan

A three-year field evaluation was conducted to assess the agronomic performance, trait associations, and diversity of 238 chickpea (*Cicer arietinum* L.) genotypes from a global collection cultivated under the semi-arid conditions of South-East Kazakhstan. The trials, carried out across three growing seasons, recorded significant variation for plant height (PH), height to lowest pod (HLP), number of lateral branches (NLB), number of seeds per plant (NSP), yield per plant (YP), and thousand-seed weight (TSW). Analysis of variance revealed significant effects of genotype origin, seed type, and year for several traits, with strong genotype × environment interactions. Correlation analysis showed that YP was strongly and positively associated with TSW (r = 0.605) and moderately with NSP (r = 0.530), while NSP and TSW were negatively correlated, indicating a trade-off between seed size and seed number. Principal component analysis (PCA) revealed that the genotypes originating from the Middle East and Africa were primarily grouped with higher values for yield per plant (YP) and thousand-seed weight (TSW), whereas South Asian germplasm showed wide phenotypic dispersion, reflecting their broad variability. Kabulitype of chickpea seeds showed a strong association with yield-related traits, while Desi types revealed greater variability and a weaker association with seed size. A total of 24 perspective genotypes, such as ICC456, ICC637, ICC1392, ICC2065, ICC3362, and ICC3410, were identified as valuable candidates for breeding aimed at improving productivity and adaptability of chickpea in South-East Kazakhstan. Overall, these results enhance understanding of the diversity and interrelationships of agronomic traits in global chickpea germplasm and emphasize the breeding potential of selected genotypes for semi-arid regions.

Keywords: chickpea, global collection, agronomic traits, semi-arid Kazakhstan.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the important legume crop, widely grown in arid and semi-arid regions due to its adaptability, nutritional value, and contribution to sustainable agriculture. Globally, chickpea occupies approximately 14.8 million hectares, with an annual production exceeding 15 million tons and an average yield of 1.01 t ha^{-1} in 2020 — considerably below the potential yield of up to 6 t ha^{-1} under optimal conditions [1]. As a member of the founder crops of the Fertile Crescent, chickpea was domesticated together with lentil (*Lens culinaris*) and pea (*Pisum sativum*) and has since spread across South Asia, the Middle East, Africa, and the Mediterranean basin, where it remains a crucial dietary protein source [2, 3].

Kazakhstan encompasses diverse agroecological zones, many of which are characterized by arid or semi-arid climates, low and variable precipitation, and temperature extremes. Agricultural production in these environments is constrained by drought stress, short growing seasons in the north, and high summer temperatures in the south [4]. Chickpea, with its deep root system, moderate water requirement, and ability to fix atmospheric nitrogen through symbiosis with *Rhizobium* spp., is well suited to these conditions and has the potential to enhance the resilience of cropping systems [5].

From an agronomic perspective, chickpea serves as a valuable rotational crop in cereal-based systems, improving soil fertility, disrupting pest and disease cycles, and enhancing sustainability [6, 7]. Economically, the rising global demand for both desi and kabuli types, particularly in South Asia, the Middle East, and expanding European markets, presents opportunities for Kazakhstan to strengthen its domestic production and explore export potential [1]. Nutritionally, chickpeas provide 18–24 % protein, complex carbohydrates, essential amino acids, minerals such as iron and zinc, and vitamins, making them a key crop for addressing food and nutrition security challenges [8, 9].

Although chickpea is a relatively recent introduction into Kazakhstan's cropping systems, research efforts over the past decade have advanced understanding of its adaptability and genetic potential under local

conditions. For example, Khasanova et al. (2021) [10] identified high-performing genotypes such as ICC-456, ICC-15697, and ICC-7272 under northern Kazakhstan environments, while Khasanova et al. (2022) [11] highlighted drought-tolerant accessions with favorable yield components. Molecular studies have also begun to support breeding programs: Mazkirat et al. (2023) [12] applied SSR (simple sequence repeat) markers to identify marker–trait associations for yield-related traits, while Ansabayeva and Akhmetbekova (2023) [13] demonstrated that biological inoculants (e.g., Baikal EM-1) enhanced yield stability under continental climatic conditions. Recent multi-location trials have identified elite cultivars with yields up to 5.94 t ha^{-1} under favorable management, underscoring the untapped potential of chickpea for Kazakhstan [14].

Despite these advances, comprehensive multi-year evaluations of large, diverse chickpea germplasm sets under Kazakhstan's heterogeneous agroclimatic conditions remain limited. Most existing studies have focused on northern environments, whereas the south-east region, characterized by warmer temperatures and distinct rainfall patterns, has received less attention. Identifying genotypes with superior yield performance, stable expression of key traits, and adaptability to such conditions is essential to broaden the genetic base of breeding materials and accelerate cultivar development.

The present study was undertaken to address this gap. Specifically, we aimed to evaluate the agronomic performance of 238 global chickpea genotypes across three consecutive growing seasons (2022–2024) in South-East Kazakhstan, assess correlations among yield and yield-related traits, and identify superior genotypes with potential utility in breeding programs targeting resilience and productivity under Kazakhstan's semi-arid agroecological conditions.

Experimental

A total of 238 chickpea (Cicer arietinum L.) genotypes were evaluated, representing diverse geographic origins including Africa (n = 26), Europe (n = 5), Latin America (n = 5), the Middle East (n = 94), and South Asia (n = 108). The collection comprised accessions of different seed types, including kabuli, desi, and pea-shaped forms, consistent with earlier global chickpea diversity assessments [15–17].Whole collection accessions were evaluated over three growing seasons (2022, 2023, and 2024)at the experimental site of LLP "Kazakh Research Institute of Agriculture and Plant Growing" (KRIAPG). Each trial was conducted in a randomized complete block design with three replications. Standard agronomic practices were followed for chickpea cultivation in each season. Traits measured: PH — Plant height (cm); HLP — Height to lowest pod (cm); NLB — Number of lateral branches (count); NSP — Number of seeds per plant (count); YP — Yield per plant (g); TSW — Thousand-seed weight (g).

Descriptive statistics (mean, maximum, minimum, standard deviation, and coefficient of variation) were calculated to assess the extent of phenotypic variation within and across geographic groups. Trait correlations were estimated using Pearson's correlation coefficient, and significance levels were determined at $p < 0.05$, $p < 0.01$, and $p < 0.001$ [18].

Analysis of variance (ANOVA) was performed using a mixed linear model to partition the effects of origin, seed type, and year, as well as their interactions, on each trait. Significance was assessed using F-tests, and post hoc comparisons were conducted where appropriate.

Multivariate analysis of trait variation was carried out using principal component analysis (PCA) to reduce dimensionality and detect patterns among traits and accessions [19]. PCA biplots were generated to visualize relationships among traits and the distribution of accessions by geographic origin and seed type, following approaches previously applied in chickpea germplasm studies.

All statistical analyses were conducted in R softwarep [20], using packages *stats*, *ggplot2*, and *factoextra* for data analysis and visualization.

Results and Discussion

Trait Variability Across Growing Seasons

The evaluation of 238 global chickpea genotypes revealed substantial variation in agronomic traits across different regions of origin (Table 1). Mean PH ranged from 29.05 cm in European accessions to 32.91 cm in those from the Middle East, with the tallest individual plant recorded in the Middle East group (46.02 cm). The HLP varied between 16.68 cm (Europe) and 18.41 cm (Middle East), with coefficients of variation (CV) generally exceeding 15 %, indicating moderate variability. The NLB per plant was highest in Middle Eastern accessions (mean 2.25) and lowest in Europe (mean 2.01), while the NSP ranged from 17.08 in European lines to 21.05 in Latin American lines, with the maximum value (32.48) also observed in Middle

Eastern germplasm. YP showed a similar trend, with the highest mean recorded in Latin America (4.43 g) and the highest individual yield in Middle Eastern lines (7.75 g).

Table 1

Descriptive statistics for agronomic traits of 238 global chickpea genotypes from five geographic origins

Traits	Max	Min	Mean	SD	CV (%)
Africa					
Plant height (cm)	36.167	25.090	30.790	3.069	9.969
Height to lowest pod (cm)	23.623	12.300	17.020	2.929	17.211
NLB — Number of lateral branches (count)	2.533	1.523	2.078	0.275	13.228
Number of seeds per plant (count)	28.667	13.450	19.043	3.377	17.734
YP — Yield per plant (g)	6.412	2.225	3.720	1.129	30.338
TSW — Thousand-seed weight (g)					
Europe					
Plant height (cm)	39.943	23.067	29.053	6.479	22.299
Height to lowest pod (cm)	21.023	14.133	16.677	2.599	15.585
NLB — Number of lateral branches (count)	2.577	1.523	2.007	0.477	23.789
Number of seeds per plant (count)	20.710	13.610	17.075	2.778	16.268
YP — Yield per plant (g)	4.661	2.161	3.202	1.077	33.640
TSW — Thousand-seed weight (g)	268.033	110.667	155.500	65.086	41.856
Latin America					
Plant height (cm)	39.477	25.333	30.189	6.344	21.014
Height to lowest pod (cm)	23.210	13.100	17.024	4.537	26.650
NLB — Number of lateral branches (count)	2.777	2.123	2.431	0.260	10.692
Number of seeds per plant (count)	24.800	17.683	21.045	2.555	12.142
YP — Yield per plant (g)	5.727	3.587	4.425	0.974	22.007
TSW — Thousand-seed weight (g)	272.050	119.983	166.637	60.715	36.436
Middle East					
Plant height (cm)	46.022	20.300	32.906	4.577	13.910
Height to lowest pod (cm)	26.743	11.567	18.410	3.269	17.758
NLB — Number of lateral branches (count)	3.357	1.410	2.250	0.346	15.382
Number of seeds per plant (count)	32.477	11.543	19.025	4.384	23.044
YP — Yield per plant (g)	7.753	1.892	4.074	1.086	26.646
TSW — Thousand-seed weight (g)	329.433	98.017	168.612	56.273	33.374
SouthAsia					
Plant height (cm)	42.007	20.500	30.574	4.438	14.515
Height to lowest pod (cm)	27.067	11.800	17.503	3.077	17.580
NLB — Number of lateral branches (count)	3.057	1.333	2.174	0.316	14.534
Number of seeds per plant (count)	31.823	11.065	19.438	4.760	24.487
YP — Yield per plant (g)	7.550	1.713	3.963	1.014	25.591
TSW — Thousand-seed weight (g)	309.350	98.767	161.850	45.121	27.878
Total					
Plant height (cm)	46.022	20.300	31.479	4.570	14.518
Height to lowest pod (cm)	27.067	11.567	17.781	3.183	17.898
NLB — Number of lateral branches (count)	3.357	1.333	2.196	0.331	15.097
Number of seeds per plant (count)	32.477	11.065	19.216	4.407	22.934
YP — Yield per plant (g)	7.753	1.713	3.974	1.061	26.686
TSW — Thousand-seed weight (g)	329.433	98.017	163.895	51.071	31.161

Note. Max — maximum observed value; Min — minimum observed value; Mean — arithmetic average; SD — standard deviation; CV — coefficient of variation

TSW exhibited the largest variability among traits, with values ranging from 98.02 g to 329.43 g. The heaviest seeds were found in Middle Eastern genotypes (mean 168.61 g), followed by Latin American lines (166.64 g), whereas European accessions had the lowest mean TSW (155.50 g). Overall, the combined dataset across all origins showed a mean plant height of 31.48 cm, height to the lowest pod of 17.78 cm, two lateral branches per plant on average, and a yield per plant of 3.97 g. These results highlight that Middle Eastern and Latin American germplasm tend to possess superior yield-related traits, whereas European and

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African lines may contribute to diversity in plant architecture. The observed variability suggests ample opportunities for selecting promising genotypes for breeding programs targeting high yield and desirable morphological attributes under South-East Kazakhstan's agro-climatic conditions.

Pearson correlation analysis explored relationships among traits, averaged across years (Fig. 3). A strong, highly significant positive correlation was observed between PH and HLP (r = 0.866; p<0.001), indicating a very close association where an increase in one trait is strongly linked to an increase in the other. Another strong positive correlation was observed between TSW and YP (r = 0.605; p<0.001), indicating that plants with larger seeds tend to produce higher yields.

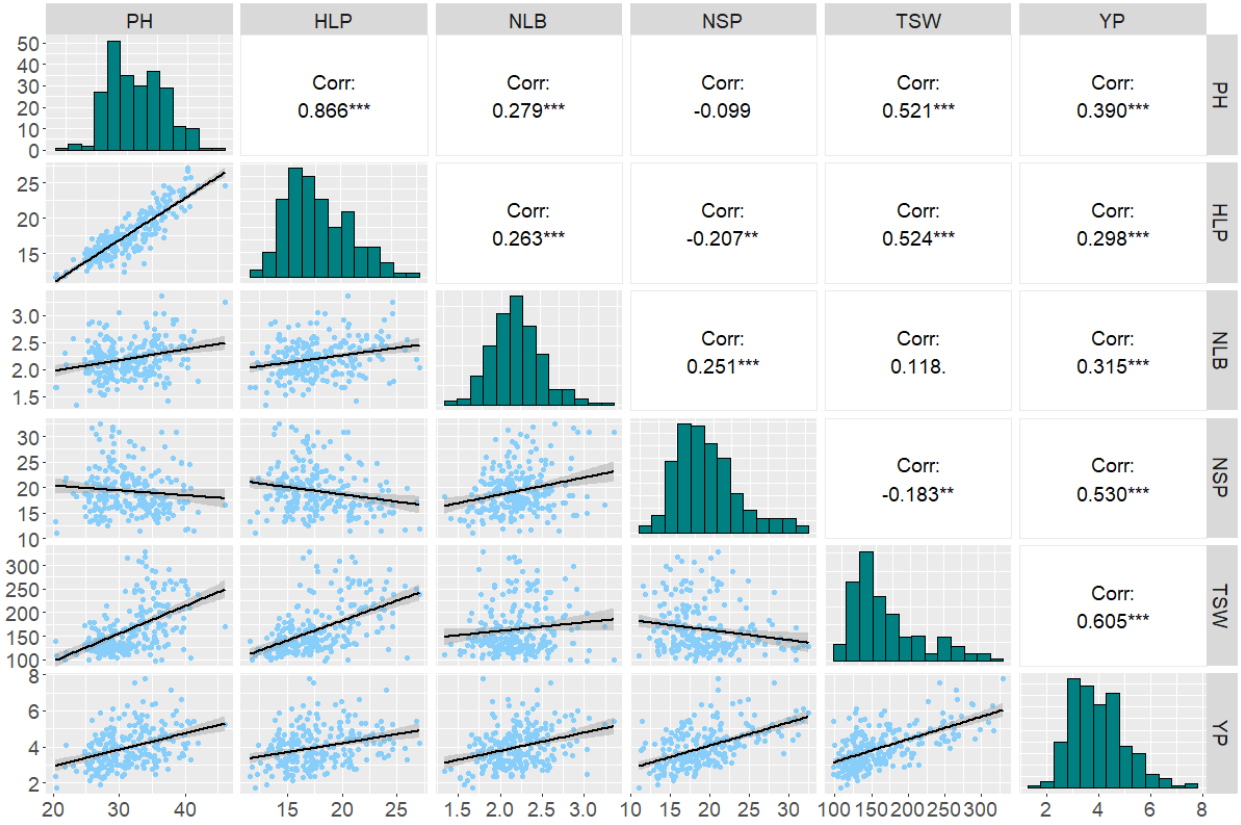

Figure 1. Correlation analysis of agronomic traits.
PH — plant height; HLP — height to lowest pod; NLB — number of lateral branches;
NSP — number of seeds per plant; YP — yield per plant; TSW — thousand seed weight

Moderate positive correlations were identified between NSP and YP (r = 0.530; p<0.001) and between PH and YP (r = 0.390; p<0.001). These suggest that while the number of seeds per pod and plant height are significantly associated with yield, their influence is less pronounced than that of thousand seed weight.

A weak but statistically significant positive correlation was noted for HLP and NLB (r = 0.263; p<0.001) and HLP and YP (r = 0.298; p<0.001). The weak negative correlation between NSP and TSW (r = -0.183; p<0.01) is also statistically significant, suggesting that there is a slight, inverse relationship between the number of seeds and their weight.

Multivariate Analysis of Accession Diversity

The analysis of variance revealed distinct effects of origin, type, and year, as well as their interactions, on several agronomic traits of the 238 chickpea genotypes evaluated (Table 2). For plant height (PH), significant main effects were detected for origin ($F = 4.291$, $p = 0.00195$), type ($F = 5.203$, $p = 0.00572$), and year ($F = 16.538$, $p < 0.001$), along with a significant origin × type interaction ($F = 3.363$, $p = 0.00522$). Other interaction terms were not significant. Similarly, height to lowest pod (HLP) was significantly influenced by origin ($F = 2.575$, $p = 0.03657$), type ($F = 5.720$, $p = 0.00344$), and origin × type interaction ($F = 2.449$, $p = 0.03262$), whereas year and higher-order interactions were not significant.

Table 2

ANOVA for agronomic traits of 238 global chickpea genotypes evaluated in South-East Kazakhstan

Traits	*Df*	*SS*	*MS*	*F*	*P value*
PH					
Origin	4	990	247,5	4,291	0,00195**
Type	2	600	300,1	5,203	0,00572**
Year	1	954	953,8	16,538	5,32E-05***
Origin: Type	5	970	193,9	3,363	0,00522**
Origin: Year	4	39	9,9	0,171	0,95317
Type: Year	2	30	14,8	0,257	0,77374
Origin: Type: Year	5	137	27,4	0,474	0,79556
Residuals	690	39794	57,7		
HLP					
Origin	4	209	52,13	2,575	0,03657*
Type	2	232	115,78	5,72	0,00344**
Year	1	5	5,34	0,264	0,60779
Origin: Type	5	248	49,56	2,449	0,03262*
Origin: Year	4	21	5,17	0,255	0,90638
Type: Year	2	12	5,81	0,287	0,75045
Origin: Type: Year	5	19	3,77	0,186	0,96775
Residuals	690	13966	20,24		
NLB					
Origin	4	3,34	0,83	2,064	0,0839
Type	2	0,57	0,28	0,7	0,4969
Year	1	173,97	173,97	430,116	<2e-16***
Origin: Type	5	2,75	0,55	1,362	0,2367
Origin: Year	4	1,01	0,25	0,625	0,6445
Type: Year	2	0,34	0,17	0,421	0,6565
Origin: Type: Year	5	2,59	0,52	1,281	0,2701
Residuals	686	277,47	0,4		
NSP					
Origin	4	154	38,6	0,474	0,755
Type	2	111	55,5	0,681	0,507
Year	1	2401	2401,1	29,465	7,92E-08***
Origin: Type	5	187	37,5	0,46	0,806
Origin: Year	4	206	51,4	0,631	0,641
Type: Year	2	319	159,4	1,956	0,142
Origin: Type: Year	5	148	29,5	0,362	0,874
Residuals	683	55659	81,5		
YP					
Origin	4	18,5	4,634	1,214	0,30367
Type	2	41,1	20,54	5,379	0,00481**
Year	1	0	0,022	0,006	0,93982
Origin: Type	5	18,2	3,641	0,953	0,4457
Origin: Year	4	12	2,99	0,783	0,53635
Type: Year	2	14,9	7,436	1,947	0,14344
Origin: Type: Year	5	10,1	2,012	0,527	0,75607
Residuals	683	2607,9	3,818		
TSW					
Origin	4	12956	3239	1,197	0,3109
Type	2	223030	111515	41,201	2,00E-16***
Year	1	102627	102627	37,918	1,26E-09***
Origin: Type	5	30225	6045	2,233	0,0494*
Origin: Year	4	1121	280	0,104	0,9813
Type: Year	2	12157	6079	2,246	0,1066
Origin: Type: Year	5	1919	384	0,142	0,9824
Residuals	686	1856715	2707		

Note. PH — plant height; HLP — height to lowest pod; NLB — number of lateral branches; NSP — number of seeds per plant; YP — yield per plant; TSW — thousand seed weight. *Df* — degrees of freedom; *SS* — sum of squares; *MS* — mean square; *F* — F-statistic; *P value* — significance level. * — $P< 0.05$, ** — $P< 0.01$, *** — $P < 0.001$

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For the number of lateral branches (NLB), year exerted a highly significant effect ($F = 430.116$, $p < 0.001$), but origin and type had no significant main or interaction effects. A similar pattern was observed for the number of seeds per plant (NSP), with year showing a strong effect ($F = 29.465$, $p < 0.001$), while origin, type, and their interactions were non-significant.

In terms of grain yield per plant (YP), type was the only factor with a significant main effect ($F = 5.379$, $p = 0.00481$), whereas origin, year, and all interaction terms were non-significant. In contrast, thousand seed weight (TSW) showed highly significant effects of type ($F = 41.201$, $p < 0.001$) and year ($F = 37.918$, $p < 0.001$), as well as a weaker but significant origin × type interaction ($F = 2.233$, $p = 0.0494$). No other interactions were significant for TSW.

Differences by Origin and Type

Principal component analysis (PCA) with origin groups revealed that the first two principal components (PC1 and PC2) collectively explained 72.1 % of the total phenotypic variation among chickpea genotypes (Fig. 2). PC1 accounted for 45.7 % of the variance and was positively associated with YP, TSW, and PH, whereas PC2, explaining 26.4 % of the variance, was positively correlated with NSP and NLB.

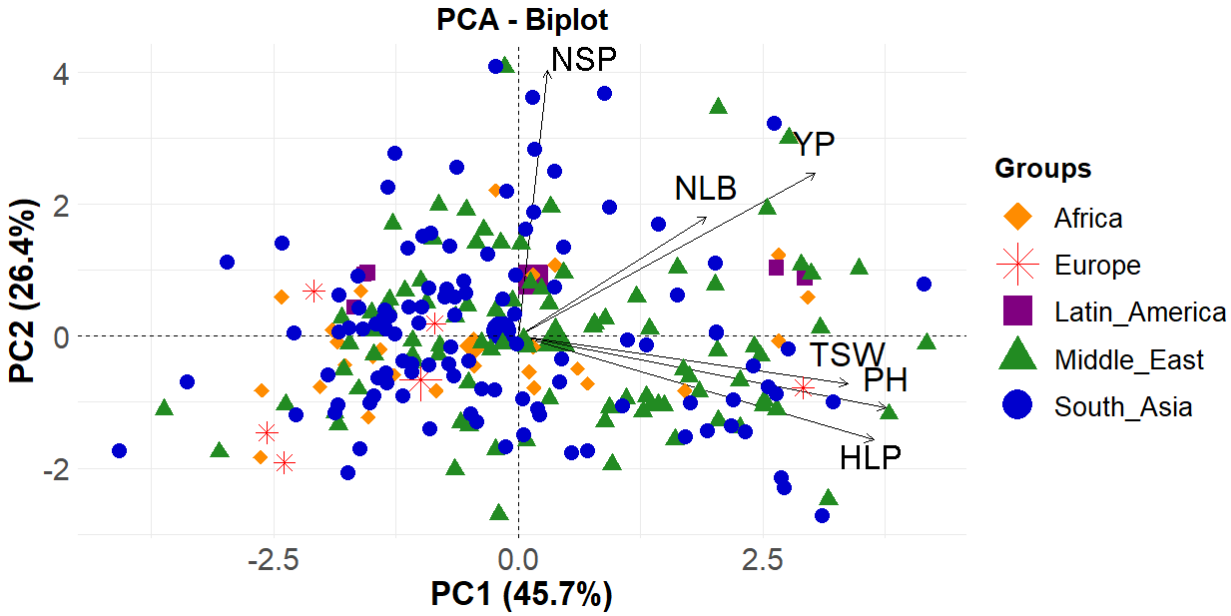

Figure 2. PCA showing the distribution of accessions from different origin groups based on six agronomic traits. PH — plant height; HLP — height to lowest pod; NLB — number of lateral branches; NSP — number of seeds per plant; YP — yield per plant; TSW — thousand seed weight

The PCA showed that chickpea accessions were partly separated by their origin. Genotypes from Africa and the Middle East were mainly grouped on the positive side of PC1, reflecting their higher YPand TSW. European genotypes clustered on the negative side of PC1, corresponding to lower performance for these traits. Latin American accessions occupied an intermediate position, representing average trait values, while South Asian accessions were widely scattered across both components, highlighting their high genetic and phenotypic diversity. Analysis of trait relationships further indicated that YP, TSW, and PH were positively associated, whereas HLP was negatively correlated with these yield components.

Principal component analysis (PCA) indicated that the first two principal components (PC1 and PC2) together explained 72.1 % of the total phenotypic variation among chickpea accessions (Fig. 3). PC1, which accounted for 45.7 % of the variance, was positively associated with yield potential (YP), thousand-seed weight (TSW), and plant height (PH).

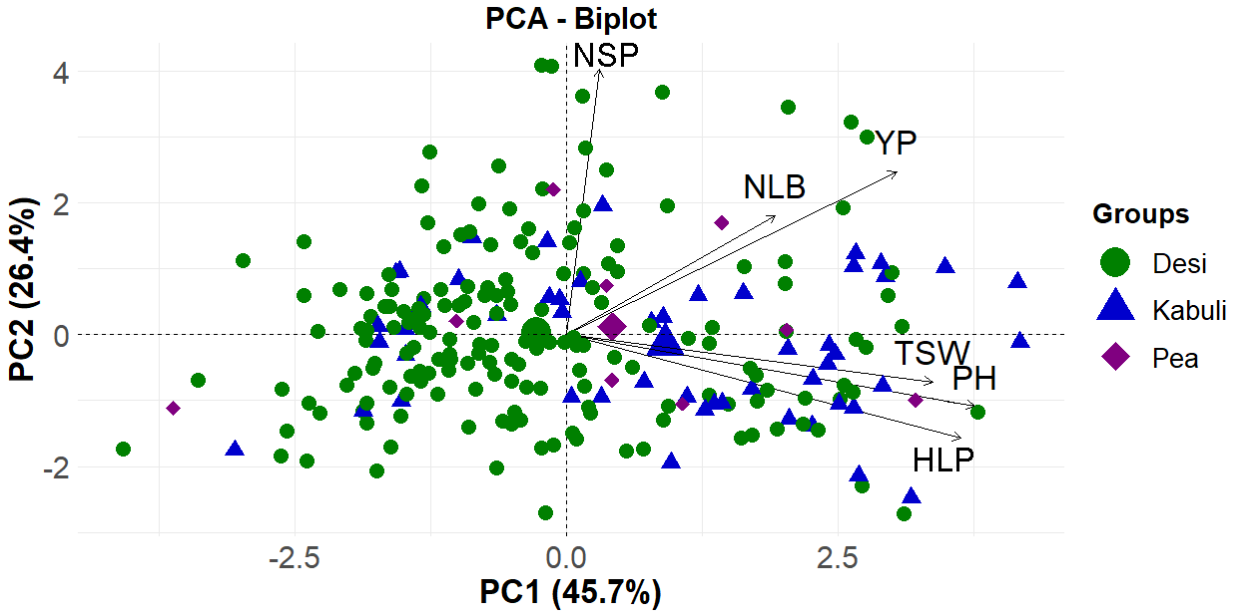

Figure 2. PCA showing the distribution of accessions from different seed types based on six agronomic traits. PH — plant height; HLP — height to lowest pod; NLB — number of lateral branches; NSP — number of seeds per plant; YP — yield per plant; TSW — thousand seed weight

PC2, explaining 26.4 % of the variance, was positively correlated with the number of pods per plant (NSP) and number of lateral branches (NLB). The PCA biplot revealed a distinct separation among the three seed types. Kabuli accessions (blue triangles) were predominantly located on the positive side of PC1, reflecting higher YP and TSW values. Desi accessions (green circles) were more widely dispersed, occupying mainly the negative and central regions of PC1, suggesting lower association with yield-related traits and greater genetic variability. Pea-shaped accessions (purple diamonds) were sparsely distributed without a clear clustering pattern, likely due to the limited number of samples.

Discussion

The present multi-year evaluation of 238 chickpea genotypes from diverse global origins under South-East Kazakhstan's semi-arid conditions revealed substantial variability in agronomic traits, emphasizing the richness of genetic diversity within the tested collection. Such diversity is critical for broadening the genetic base of local chickpea breeding programs, which remain relatively narrow compared to major chickpea-growing regions [21, 22]. The significant main effects of genotype origin and seed type on PH, HLP and TSW demonstrate that both genetic background and seed morphology strongly influence performance under local environments. Conversely, strong year effects for NLB and NSP highlight the impact of annual climatic variation on yield-related traits, a finding consistent with other multi-environment studies in chickpea [23, 24].

The strong positive association between YP and TSW (r = 0.605) indicates that larger-seeded genotypes, particularly kabuli types, represent promising candidates for yield improvement in South-East Kazakhstan. This is in line with earlier findings that seed size is one of the primary determinants of market-preferred yield gains [25, 26]. The moderate correlation between YP and NSP (r = 0.530) suggests that increasing seed number can also contribute to yield gains, although the negative correlation between NSP and TSW reflects a well-known trade-off between seed size and seed number in grain legumes [27]. Thus, breeding strategies should seek to optimize both traits, potentially through ideotype-based selection or marker-assisted introgression of favorable alleles.

PCAprovided further insights into the structuring of diversity. Accessions from the Middle East and Africa clustered toward higher yield-related traits, supporting their utility as donor parents for productivity improvement. This agrees with the historical role of the Fertile Crescent and surrounding regions as hotspots of chickpea diversity [17]. In contrast, European accessions generally exhibited lower yield potential but may serve as sources of unique alleles for plant architecture or phenology. Latin American genotypes showed intermediate performance, whereas South Asian lines displayed wide dispersion, reflecting both the high genetic variability and broad adaptation of germplasm from this major chickpea-growing region [28]. The dif-

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ferentiation of kabuli and desi types in the PCA biplot underscores their distinct breeding potential, with kabuli types aligning more strongly with yield-related traits.

Importantly, the identification of 24 superior genotypes across years, including ICC456, ICC637, ICC1392, ICC2065, ICC3362, and ICC3410, provides valuable immediate resources for chickpea improvement in Kazakhstan. Their consistent performance under variable seasonal conditions suggests they possess both yield potential and adaptability. Integrating these genotypes into local breeding programs can enhance productivity and stability under semi-arid environments, where water limitation and temperature stress are key challenges [29].

Conclusion

This study demonstrated that substantial genetic variation exists among 238 chickpea genotypes when cultivated under South-East Kazakhstan conditions. Yield potential was strongly linked to seed size and moderately to seed number, indicating that these traits should be key targets in future breeding programs. PCA and ANOVA analyses confirmed that geographic origin and seed type play an important role in improving agronomic traits, while strong year-to-year variation underlines the need for multi-season testing of genotypes.

Identified high-yielding and stable genotypes from the Middle East and Africa, offering valuable sources to expand the genetic base of local breeding programs. Kabuli types stood out for their close association with yield components, making them especially promising for yield improvement. The 24 perpective lines identified in this study represent an important resource for developing chickpea cultivars with improved productivity and resilience in the semi-arid regions of Kazakhstan.

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Conflict of Interest

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. **Zatybekov A.K.** – Investigation, Methodology, Writing-original draft preparation, Writing-review & Editing; **Yeshengaliyeva A.N.** – Writing-review & Editing; **Anuarbek Sh.N.** – Funding acquisition, Writing-review & Editing; **Kudaibergenov M.S.** – Resources, Data curation; **Turuspekov Y.K.** – Cconceptualization, Plant material collection; **Abugalieva S.I. —** Conceptualization, Writing-review & Editing.

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Қазақстанның оңтүстік-шығысында өсірілген 238 әлемдік ноқат (*Cicer arietinum* L.) генотиптерін далалық бағалау және әртүрлілігі

Қазақстанның оңтүстік-шығысының жартылай шөлейтті жағдайында өсірілетін әлемдік коллекциядан 238 ноқат генотипінің (Cicer arietinum L.) агрономиялық көрсеткіштеріне, сипаттамаларының байланысына және әртүрлілігін бағалау үшін үш жылдық дала зерттеуі жүргізілді. Үш вегетациялық маусымда жүргізілген сынақтар өсімдіктің биіктігі (ӨБ), төменгі бұршақтың бекітілу биіктігі (ТББ), бүйірлік бұтақтардың саны (ББС), өсімдіктегі бұршақ саны (ӨБС), өсімдік өнімділігі (ӨӨ) және 1000 тұқымның салмағы (МТС) бойынша айтарлықтай өзгергіштікті анықтады. Дисперсиялық талдау бірнеше белгілер бойынша генотиптің шығу тегінің, дән түрінің және жылдың маңыздылығын көрсетті, сондай-ақ генотип × қоршаған орта ықпалдарының күшті әсерін анықтады. Корреляциялық талдау ӨӨ мен МТС арасында күшті және оң корреляция (r = 0,605) және ӨБС мен МТС арасында орташа оң корреляция (r = 0,530) бар екенін көрсетті, ал ӨБС-де теріс корреляция анықталды, бұл дәннің көлемі мен саны арасында ерекшеліктер бар екенін көрсетеді. Негізгі компоненттерді талдауда алғашқы екі компоненттің жалпы вариацияның 72,1%-ын түсіндіретінін көрсетті: Таяу Шығыс пен Африка елдерінен алынған үлгілер жоғары ӨӨ және МТС мәндеріне топтастырылды, ал оңтүстік Азиядан келген генотиптер кең фенотиптік дисперсиямен сипатталды. Кабули типі жалпы өнімділік белгілерімен байланысты болды, ал дези-типі тұқым өлшемінің үлкен өзгергіштігімен әлсіз байланысты көрсетті. Қазақстанның оңтүстік-шығысы жағдайында ноқаттың өнімділігі мен бейімделуін арттыру үшін перспективалы селекциялық материалды ұсынатын ICC456, ICC637, ICC1392, ICC2065, ICC3362 және ICC3410 сияқты 24 жоғары өнімді желі анықталды. Алынған нәтижелер ноқаттың әлемдік гермоплазмасы белгілерінің генетикалық әртүрлілігі мен өзара байланысы туралы құнды ақпарат береді және жартылай шөлейт аймақтарға бағытталған селекциялық бағдарламаларда пайдалану үшін таңдалған генотиптердің әлеуетін көрсетеді.

Кілт сөздер: ноқат, әлемдік үлгілер, агрономиялық белгілер, жартылай шөлейтті Қазақстан.

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Полевая оценка и разнообразие 238 мировых генотипов нут (*Cicerarietinum* L.), выращенных в Юго-Восточном Казахстане

Проведена трёхлетняя полевая оценка агрономических признаков, их взаимосвязей и разнообразия 238 генотипов нута (*Cicerarietinum* L.) из мировой коллекции, возделываемых в полузасушливых условиях юго-востока Казахстана. Испытания, проведённые в течение трёх вегетационных сезонов, выявили значительную вариабельность по высоте растения (ВР), высоте прикрепления нижнего боба (ВПНБ), числу боковых ветвей (ЧБВ), числу бобов с растения (ЧБР), урожайности с растения (УР) и массе 1000 семян (МТС). Дисперсионный анализ показал достоверное влияние происхождения образца, типа семян и года на ряд признаков, а также выявил выраженные взаимодействия генотип × среда. Корреляционный анализ продемонстрировал сильную положительную связь УР с МТС (r = 0,605) и умеренную — с ЧБР (r = 0,530), тогда как между ЧБР и МТС выявлена отрицательная корреляция, отражающая известный компромисс между размером семян и их количеством. Анализ главных компонент показал, что первые две компоненты объясняют 72,1 % общей вариации: образцы из стран Ближнего Востока и Африки группировались в сторону высоких значений УР и МТС, тогда как генотипы из Южной Азии характеризовались широкой фенотипической дисперсией. Кабули-тип в целом был ассоциирован с урожайными признаками, тогда как дези-тип демонстрировал большую изменчивость и слабую связь с размером семян. Выделены 24 высокопродуктивные линии, включая ICC456, ICC637, ICC1392, ICC2065, ICC3362 и ICC3410, представляющие перспективный селекционный материал для повышения урожайности и адаптации нута в условиях юго-востока Казахстана. Полученные результаты предоставляют ценную информацию о генетическом разнообразии и взаимосвязи признаков мировой гермоплазмы нута, а также подчёркивают потенциал отобранных генотипов для использования в селекционных программах, ориентированных на полузасушливые регионы.

Ключевые слова: нут, мировая коллекция, сельскохозяйственные признаки, полузасушливый Казахстан.

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Study of the distribution of Linaria cretacea in Kazakhstan and neighboring countries: data from an electronic herbarium

Linaria cretacea Fisch. ex Spreng., commonly known as chalk toadflax, is a rare calciphilous species in the family Plantaginaceae, listed in the Red Data Books of Kazakhstan and Ukraine. Here, we present a synthesized overview of its distribution derived from digitized herbarium records (Kazakhstan, Russia, Ukraine) and recent field surveys in the Aktobe Region, alongside an ecological characterization of its chalk-derived habitats. The species' range is highly disjunct, spanning eastern Ukraine, the southern European part of Russia (Don River basin), and the far northwestern corner of Kazakhstan. Most populations occur on exposed, rubbly chalk slopes—so-called "chalk mountains." We analyzed specimen data and observations by region and year, producing summary maps and distribution tables. In Kazakhstan, only a handful of isolated populations in the Aktobe Region have been confirmed by up-to-date collections and in situ observations. Key habitat features include calcium-rich substrates, continental steppe climate, and a specialized chalk flora. We discuss the low population densities and high vulnerability of *L. cretacea* owing to its narrow ecological niche and anthropogenic threats—chalk quarrying, livestock grazing, and successional overgrowth. The current conservation status across its range is reviewed, and recommendations for population monitoring and protection are offered. We conclude by emphasizing the scientific importance of chalk ecosystems and the urgent need to safeguard these refugia for relict plant species.

Keywords: Linaria cretacea, distribution, Kazakhstan, electronic herbarium, field research, vulnerability, monitoring, plant protection.

Introduction

Significance of Studying Linaria cretacea

Linaria cretacea Fisch. ex Spreng. is a perennial herb of the Plantaginaceae (formerly Scrophulariaceae), strictly confined to chalk outcrops. Its relictual range, centered around the eastern Black Sea region, makes it of high conservation concern. The species features in the national Red Data Books of Kazakhstan and Ukraine, as well as in various regional conservation lists within the Russian Federation. Its restricted habitat—where Upper Cretaceous chalk emerges at the surface—combined with ongoing human pressures, renders *L. cretacea* particularly susceptible to local extirpations. Consequently, a detailed understanding of its distribution, population dynamics, and habitat requirements is essential to formulate effective preservation strategies.

Geographic and Taxonomic Context

The natural range of *L. cretacea* is fragmented into three main clusters: (1) the eastern Ukrainian chalk ridges of Luhansk and Donetsk oblasts; (2) disjunct pockets across the southern European Russian steppes (notably in Belgorod, Voronezh, Saratov, Volgograd, and Rostov oblasts, with outliers in Ulyanovsk Oblast); and (3) the westernmost limit in the C is Ural region (Orenburg Oblast) extending into northwestern Kazakhstan (Aktobe Region). This patchwork distribution spans a broad east‑west and north‑south gradient, reflecting both geological constraints (chalk exposed in "island" outcrops) and historic biogeographical processes. Systematically, *L. cretacea* belongs to the genus *Linaria Mill*., with earlier synonyms such as Linaria creticola Kuprian now consolidated under the accepted name *L. cretacea*.

Objectives

The present study aims to collect and analyze up-to-date information on the occurrence of *L. cretacea* in Kazakhstan and neighbouring countries with the following specific objectives:

- To collect occurrence records from electronic herbarium databases and existing literature.

- To supplement these historical data with results of targeted field studies, especially in the Aktobe region of Kazakhstan.
- To map and tabulate the geographical distribution of the species by region and collection date.
- To characterize the ecological and geomorphological conditions of its habitats on chalk outcrops.
- To assess the current status of known populations and identify the main threats.
- To propose practical recommendations for the ongoing monitoring and conservation of *L. cretacea*.

Materials and methods of research

Herbarium Data Sources

To delineate the distribution of *L. cretacea*, digitized specimen records from several major online herbariums were used: the National Herbarium of Kazakhstan (Institute of Botany and Phytointroduction, Almaty) provided collections from western Kazakhstan; the Herbarium of the Komarov Botanical Institute (LE, St. Petersburg) and the Herbarium of Moscow State University (MW) provided material covering European Russia and the Cis-Urals; and Ukrainian collections (Kholodny Institute of Botany, NASU — KW — and various university herbaria) provided finds from eastern Ukraine. For each digital record, label information was extracted, including the modern administrative location, geographic coordinates, collection date, repository, and collector's name. In total, about fifty *L. cretacea* specimens dating from the late 19th to early 21st centuries were studied across the species' range [1].

Field Surveys and Observations

Particular attention was paid to current locations in Kazakhstan. In 2023, a study of natural cenopopulations of *L. cretacea* was conducted in the natural population of the species in the Ishkargantau chalk massif (Fig. 1).

Figure 1. Ishkargantau chalk massif (photo by the author)

GIS Analysis.

A geodatabase of *L. cretacea* occurrence points was compiled.

Habitat and ecology data processing

Habitat characterization was based on field notes and published ecological reports. For each site, substrate type (hard chalk, shingle chalk, or marl), slope aspect and gradient, evidence of recent erosion or vegetation cover, and degree of disturbance were recorded.

Conservation status assessment

Based on national and regional Red Data Books and recent botanical literature, the rarity categories assigned to *L. cretacea* across its range were reviewed, and the populations occurring within protected areas were identified. The situation in Kazakhstan has been studied in detail: despite its inclusion in the national

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Red Book, no formal protection measures currently protect the known habitats of this species in the Aktobe region, which highlights the urgent need for special conservation measures.

Description of the data set

L. cretacea occurrence table in Darwin Core format (occurrence.csv) with the following fields: occurrenceID, scientificName, eventDate, country, stateProvince, locality, decimalLatitude, decimalLongitude, basisOfRecord, etc.

Link: https://github.com/olessya-max/linaria-cretacea.git

Results and discussions

Regional distribution of Linaria cretacea

The known localities of *L. cretacea* form a highly fragmented range concentrated in three main areas:

Eastern Ukraine — mainly in the Luhansk and Donetsk regions, corresponding to the chalk-rich Seversky Donets River catchment and adjacent chalk ridges;

Southeastern European Russia — primarily the Don River basin and the lower Volga-Don interfluve (Belgorod, Voronezh, Rostov, Volgograd, Saratov regions), with distant records extending north to the Ulyanovsk region;

The Cis-Urals and Northwestern Kazakhstan — chalk outcrops of the Orenburg region (Russia) and the adjacent Aktobe region (Kazakhstan).

In each of these zones, *L. cretacea* is associated with steppe landscapes wherever chalk outcrops pierce the surface. The composite map (Fig. 2) displays all verified localities taken from literature, herbarium specimens, and fieldwork.

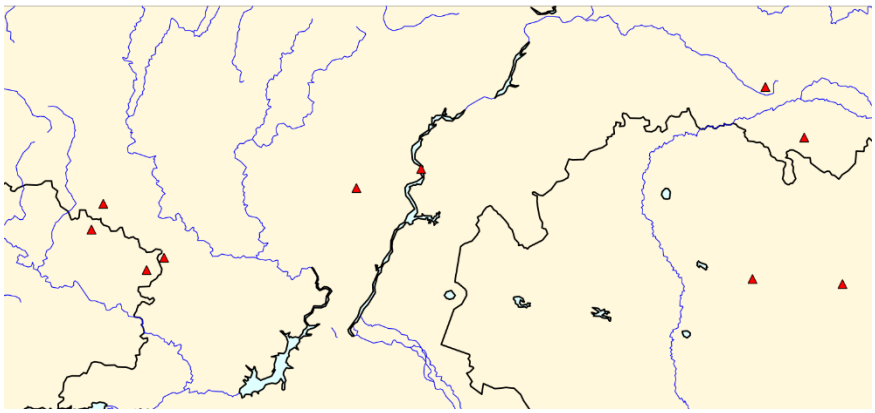

Figure 2. *Linaria cretacea* distribution: triangles marking the species locations in Eastern Europe and Northwestern Kazakhstan

In Ukraine, *L. cretacea* is found exclusively in the Far East of the country. Classic localities include the environs of Slavyansk and Svyatogorsk (Donetsk Oblast) and the chalk outcrops near Streltsovka (Meloevsky district, Luhansk Oblast), in particular the “Streltsovka steppe”. These localities provided the original specimens on which Fischer and Sprengel based their 1825 description. Recent field visits (e.g. 2017) have confirmed its persistence on the chalk cliffs around Streltsovka. Although some populations have been degraded by industrial development and regional conflicts, *L. cretacea* remains listed in the 2009 Red Data Book of Ukraine as a vulnerable species, known only from these two areas.

Throughout southern and central European Russia, *L. cretacea* occurs in scattered colonies. The northernmost stands are in Belgorod and Voronezh Oblasts, marking the upper boundary of the Cretaceous steppe flora. In Belgorod (e.g. Rovensky District, Nagolnoye village), field confirmations date back to no later than 2019. In Voronezh, there are several historical sites (Liski, Rossoshansky, Kantemirovsky Districts) with herbarium material covering the 1930s–1980s. To the south, along the Don River, the species occupies chalk hills in Rostov Oblast and on river banks in Volgograd Oblast (Olkhovsky, Kalachevsky Districts). In Saratov Oblast, it has been known from the Khoper and Medveditsa valleys since the 1990s, and the only record from the early 20th century exists on the right bank of the Bityug River in Ulyanovsk Oblast. Each of these regions includes *L. cretacea* in its own Red Book (categories from II — decreasing numbers, to III — rare).

Although population sizes are generally small and declining, some clusters (for example, along the Don in the south of Voronezh and north of Rostov regions) still form locally abundant stands.

Orenburg region is the easternmost outposts of the Eurasian distribution of the species. Here, two key chalk massifs — Sol-Iletsky (Upper Chebendinsky Mountains) and Perevolotsky (Chesnokovsky Hills) — yielded records from the mid-2000s: herbarium collections in 2008 and 2015 on the slopes of the Chesnokovsky Ridge and field observations in 2016 on the Upper Chebendinsky Ridges. These steppe semi-desert outcrops are floristically distinct from European sites, but demonstrate the species' tolerance of more continental climates. *L. cretacea* is classified as rare (category III) in the regional Orenburg Red Book, with explicit calls for population monitoring [2].

In Kazakhstan, *L. cretacea* reaches its eastern limit, today bounded by the northwest Aktobe Region. The following chalk localities have been studied:

Akyrap. The Ishkargantau chalk ridge is located southwest of the village of Akyrap, Kobdinsky District, Aktobe Region (about 15–17 km) and 70–80 km northwest of the village of Kursay. The chalk ridge consists of high cliffs and deep ravines rising separately from the surrounding plains.

Two chalk massifs in the Khobdinsky District, Aktobe Region, should be designated as natural monuments. One of them, Mount Shangrou, is located 15 km west of the village of Akrab, on the left bank of the Bolshaya Khobda. The other, Mount Itas, or Zhantyztau, is located 13 km southwest of the village of Novonayezhdinkiy in the upper reaches of the Kil River.

The Aktolagay Ridge is located in the Baiganinsky District. The length of the mountain range is 90 km, the width is 5–10 km, and its highest point is Mount Kiyakty (217 m). The difference in altitude on the plateau ledge reaches more than 130 m. It connects with the Caspian Lowland along the bank of the Zhem River. The ridge is composed of chalk rocks.

Bestau is a chalk mountain that occupies the east and northeast of the village, 40–45 km from the village of Kobda. It is located approximately 4-5 km south of the nearest rural district of Bestau (former Pyatigorka). Consequently, in Kazakhstan, *L. cretacea* is highly localized, and its total national range covers only a few tens of square kilometers. First listed in the Red Book of the Kazakh SSR in 1981 and assigned to Category II (rare) in the Red List of Kazakhstan in 2006, none of these remnant populations currently fall within any of the protected areas [3–5].

Table 1

Analysis of the electronic herbarium of the distribution of *Linaria cretacea*

Region	Number of samples	Main locations	Collection date	Data source	Notes
Kazakhstan	15	Aktobe region, Uralsk, Kostanay, Pavlodar	1950-2020	Institute of Botany and Phytointroduction	The most complete data on the distribution of the species.
Russia	36	Southern Urals, Siberia, Altai	1955-1990	Electronic Herbarium of the Kyrgyz National Academy of Sciences	Limited number of specimens, requires clarification.
Ukraine	18	Kharkiv region, Lugansk region,	1904-1970	Russian Academy of Sciences, Moscow State University	
General information	69	-	-	-	Need for further research.

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Table 2

Additional data on herbarium specimens from the electronic herbarium of the Institute of Botany and Phytointroduction»

Family	Genderindex	View	Region	Collection (label)	Date	Collectorsanddeterminants
Scrophulariaceae	7480Linaria	Linaria cretaceaFisch.ex Spreng.	Spurs of the Common Syrt	Ural region Okr.g. Uralsk.	01.06.1925	Poyarkova T. F.
Scrophulariaceae	7480Linaria	Linaria cretaceaFisch.ex Spreng.	Embinsky	Adaevsky u. Between the river Amboy and Ust-Urtom. Chalk outcrops between Kopty-Kuduk and Khan-dyurt-kul.	14.06.1926	Rozhevits R. Yu., Ilyin M. M., Avramchik M. N.
Scrophulariaceae	7480Linaria	Linaria cretaceaFisch.ex Spreng.	Embinsky	Adaevsky u. Between the river Amboy and Ust-Urtom. G. Burlyu-pak on the river. Embe. Chalkoutcrops.	29.06.1926	Krasheninnikov I. M.
Scrophulariaceae	7480Linaria	Linaria cretaceaFisch.ex Spreng.	Embinsky	Adaevsky u. Between the river Amboy and Ust-Urtom. ChalkmountainAk-tau.	10.06.1926	Rozhevits R. Yu., Ilyin M. M., Avramchik M. N.

Digital herbarium analysis shows that *L. cretacea* (Fig. 3) is quite widespread in Kazakhstan, especially in the Aktobe region, where most of the specimens are concentrated. This is supported by data collected from various sources, including herbarium collections, which indicate the presence of stable populations in this region. For example, in the Aktobe region, 15 specimens were recorded, collected between 1950 and 2020, which highlights the importance of this region for the conservation of this species [6, 7]. Also, the studies of Aubakirova, Baytenov and others demonstrate the use of data from herbariums for a more detailed study of the flora and fauna of Kazakhstan [8, 9].

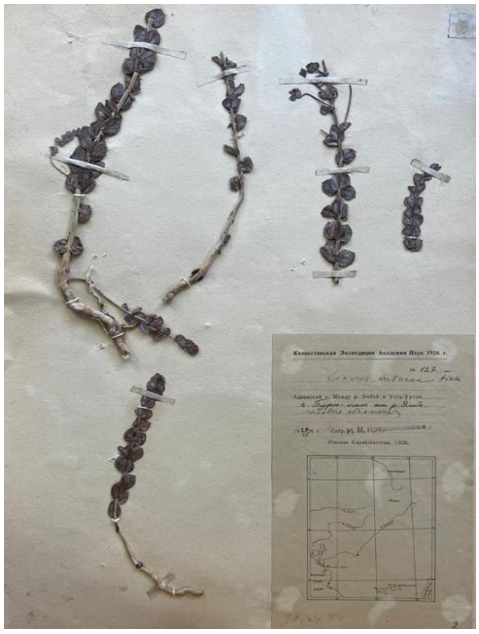

Figure 3. Herbarium of Linaria cretacea

Ecological characteristics of habitats

Landforms and soils. *L. cretacea* is an obligate calciphile, associated with outcrops of Upper Cretaceous chalk and marl. It thrives on steep, often south-facing, chalk escarpments where the substrate is either bare

rock or a thin layer of humus over chalk rubble, an extreme edaphic environment with very high calcium carbonate content. The species is absent from areas where continuous turf has formed on the chalk; it prefers fresh erosional landforms such as scree slopes, gully walls, and isolated outcrop hills. In the Cis-Urals and northwest Kazakhstan, these chalk ridges — locally known as "chalk mountains" — rise 150–200 m above the surrounding semi-desert steppe plain. A typical habitat with scattered individuals on an open chalk slope is shown in Figure 4.

Figure 4. Chalk toadflax (*Linaria cretacea*) on an open chalk slope, ID:44-228-5009 [10]

Climate. All known populations of *L. cretacea* inhabit the conditions of moderate continental steppe climate. Winters are cool (average January temperature fluctuates from –5 °C in the southern sectors to –15 °C in the north), and summers are hot (average July temperature from +22 to +25 °C). Annual precipitation decreases from west to east — from 450–500 mm in Ukraine and western Russia to 300–350 mm in the foothills of the Urals and Aktobe region — often falling in the form of irregular heavy showers. Due to their height and white substrate, chalk ridges are intensely heated by direct sunlight and are subject to rain erosion. *L. cretacea* demonstrates xerophytic adaptations: a glaucous leaf surface and a short growing season (flowering in June–July followed by partial death of the above-ground parts). In dry springs the number of flowering shoots may be sharply reduced; the plant survives by means of rhizomes and recovers when moisture conditions improve. Thus climatic limitations act in combination with substrate sparseness to limit the distribution of the species.

Plant community context. In each locality *L. cretacea* occurs in chalk steppe communities — petrophyll grasslands dominated by calciphilous forbs and dwarf shrubs. Common associate species include Euphorbia cretophila (chalk spurge), Polygala cretacea (chalk spurge), Matthiola fragrans (fragrant rootstock), Gypsophila paniculata (paniculate gypsophila) and low shrubs such as Artemisia salsoloides and cushion plants such as Nanophytonerinaceum. On more stable grassy chalk plateaus (with tussocks of Stipa capillata and Astragalus spp.) *L. cretacea* is displaced and absent. Its optimum occurs on unstable screes and fresh eroding slopes, where it is one of the few perennials able to establish near small shrubs and annuals. The complete absence of soil (vertical chalk surfaces) is also unsuitable; the species prefers small terraces or benches that capture enough substrate for rooting. The texture — fine chalk rubble — is crucial for seedling establishment and vegetative regeneration. These consistent edaphic requirements throughout its range emphasize the narrow specialization of the species to chalk ecotopes [11].

Population Size and Trends

Across its entire range, *L. cretacea* exists in small, discrete populations, each typically comprising only a few to a few dozen individuals on sites of a few hundred square meters to a hectare. For example, most colonies in Belgorod and Voronezh oblasts consist of 10–30 flowering shoots—and rarely up to 100—while Orenburg Oblast populations number 20–50 plants. In Kazakhstan, each known site contains no more than around 50 specimens. Detailed morphometric studies of four populations in Aktobe Region reveal similar demographic structures: a dominance of mature, reproductive individuals, limited recruitment of seedlings,

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and an overall density of only 2-3 plants per 10 m². These data suggest stable but low reproductive output, with population sizes remaining at a consistently low plateau.

Historical comparisons indicate either stable low numbers or outright declines since the mid‑20th century, likely driven by habitat destruction and successional change. In Rostov Oblast, several 1980s sites have not been relocated in 2000s surveys—probably lost to slope exploitation or shrub encroachment. In Voronezh Oblast, once-common stands along the Toluchëevka and Bityug rivers now survive as mere isolated clumps. In Luhansk and Donetsk oblasts, industrial land use and regional conflicts have further contracted the species' footprint. Nonetheless, some strongholds—such as the Don valley border between Voronezh and Rostov oblasts, and the Streltsivska Steppe reserve in Luhansk—still harbor hundreds of individuals, serving as refugia for chalk flora.

Long-term monitoring in protected sites is rare but encouraging: in the Belogorye Nature Reserve (Belgorod Oblast), a chalk slope population of approximately 50 plants has shown no decline over two decades of observation. Such cases remain exceptional. The prevailing pattern is one of acute vulnerability and potential extirpation of local populations absent ongoing management and monitoring.

Limiting Factors and Threats

Substrate Specialization. The primary natural constraint on *L. cretacea* is its strict dependence on pure chalk substrates. The species cannot establish on any soil type other than freshly exposed Cretaceous chalk, which confines its distribution to isolated "islands" of chalk outcrops. Even within these sites, it occupies only very specific microhabitats—rocky scree slopes with minimal plant competition. This extreme habitat specialization fragments its range into discrete patches, effectively isolating populations and limiting gene flow. Such isolation fosters genetic differentiation among colonies and may reduce their resilience to environmental stress.

Livestock Grazing and Trampling. Although chalk slopes are generally unsuitable for agriculture, they are often used informally as summer grazing grounds. Cattle and sheep are herded along narrow trails across chalk outcrops, causing direct mechanical damage to plants and accelerating soil erosion. A single herd can wipe out an entire small colony, since *L. cretacea* grows in tight patches. Hoof compaction also degrades the fragile chalk substrate, impeding seedling establishment and further diminishing recruitment. Grazing pressure thus contributes significantly to population declines, especially where no formal protection exists.

Chalk Quarrying. Chalk is a valuable raw material for construction and industry, and many chalk hills have been exploited by quarry operations. In quarries, vegetation cover is completely removed, eradicating all local populations. For instance, in Donetsk Oblast, several historic *L. cretacea* sites were destroyed by mid‑20th‑century mining. While active extraction has not yet impacted the known populations in Kazakhstan and Orenburg Oblast, future development plans for these resources pose a potential risk to the remaining colonies.

Successional Overgrowth. Where grazing or quarrying cease, open chalk slopes often undergo natural succession. Pioneer shrubs (e.g., Caragana frutex, Rosa spinosissima) and turf‑forming grasses gradually colonize eroded scree, shading out *L. cretacea*. As these microhabitats stabilize and lose their bare-rock character, suitable niches vanish. Paradoxically, limited natural disturbance—minor rock falls or ephemeral erosion—is essential to maintain the sparse, open conditions that *L. cretacea* requires. Both cessation and intensification of disturbance (through uncontrolled grazing) can upset this balance and lead to habitat loss.

Conservation Status and Current Measures. *L. cretacea* is legally protected at national and regional levels across its range. It is classified as Rare or Vulnerable in the Red Data Books of Ukraine and Kazakhstan and appears in the regional Red Data Books of at least eight Russian oblasts. Some populations occur within existing protected areas—such as Belogorye Nature Reserve (Belgorod Oblast), the Toluchëevka River steppes (Voronezh Oblast), and several nature monuments in Luhansk Oblast. However, most colonies lie outside any formal conservation unit. In Kazakhstan, none of the chalk ridges harboring the species have protected status, a gap highlighted by recent studies as a serious threat even to currently stable populations. Immediate action is needed to initiate monitoring and protective measures.

Recommendations for Monitoring and Conservation

Long‑term Population Monitoring. Establish fixed monitoring plots at each known *L. cretacea* site. Conduct systematic counts of individuals, record demographic structure, and assess habitat condition annually or every 2-3 years. In Kazakhstan, these plots should be set on the Ishkargantau and Akshatau ridges; in

Orenburg Oblast on the Upper Chebendinsky and Chesnokovsky hills; and in Ukraine and Russia at key chalk reserves and steppes. Local botanical institutions and universities should be enlisted for ongoing data collection and analysis.

Designation of New Protected Areas. Expand the network of conservation reserves to include the most important chalk ecosystems supporting *L. cretacea*. In Aktobe Region, Kazakhstan, confer "nature monument" status on one or more chalk ridges (e.g., Ishkargantau, Akshatau) to secure legal protection against mining and unmanaged grazing. Similarly, in Orenburg Oblast, establish a dedicated zakaznik (protected landscape) on the Chesnokovsky chalk hills, where multiple rare chalk-specialist species co‑occur. Existing reserves in Russia and Ukraine should have their management plans reviewed and amended to prohibit any slope-disturbing activities.

Regulation of Grazing and Quarrying. For populations outside protected zones, engage local herders and land users in management plans. Restrict livestock access to critical slopes during the species' growing season (spring–summer) by creating alternative herding routes. Legally ban any new chalk mining within known *L. cretacea* habitats and direct extraction to less biodiverse areas. Public information campaigns—including informational signage at trailheads—should raise awareness of the species' protected status and the importance of preserving chalk ridges.

Ex Situ Conservation and Reintroduction Research. Seed collection and cultivation experiments should be initiated in regional botanical gardens (e.g., Voronezh State University, Almaty Botanical Garden, Astana Scientific Center). Maintaining living ex situ populations will safeguard the genetic diversity of *L. cretacea* and allow for propagation trials. Parallel development of reintroduction protocols—using restored chalk spoil slopes or artificially created scree terraces—can pave the way for population reinforcement or reestablishment at sites where the species has been extirpated. Although technically challenging, these measures offer a promising route to bolster in situ conservation outcomes.

Conclusion

Linaria cretacea is an example of a relict species of the Cretaceous flora of southeastern Europe and adjacent Asian regions. Our consolidated analysis shows that, despite its fragmented distribution and declining numbers, the species persists in isolated "islands" of the Cretaceous steppe from the Luhansk region of Ukraine, parts of Eastern Russia and its Orenburg region, and the Aktobe region of Kazakhstan. With appropriate conservation measures, Cretaceous toadflax can maintain viable populations. From a scientific point of view, *L. cretacea* is invaluable as an indicator of specialized calciphile communities and as a model for studying the evolution of narrow endemics, their morphological plasticity and genetic diversification. Moreover, it significantly enriches the biodiversity of steppe ecosystems, which are particularly vulnerable. Its chalk habitats serve as refuges for numerous rare taxa and provide insight into the evolutionary history of the Eurasian flora. Conservation of *L. cretacea* is of both national and international importance, as its range crosses many borders. Continued collaboration between botanists and ecologists from Ukraine, Russia and Kazakhstan is essential for coordinated monitoring and protection efforts. Implementation of the recommended measures—from the establishment of new protected areas to public awareness campaigns—will ensure the long-term survival of *L. cretacea* and preserve these unique "white mountains" of the steppe for future generations.

Author Contributions

The author is solely responsible for the conception, conduct of the study, data analysis, writing of the article and all aspects of publication.

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М. Бакытжанова

Linaria cretacea-ның Қазақстанда және оған жақын елдерде таралуын зерттеу: электронды гербарий деректері

Мақалада электронды гербарий деректеріне баса назар аудара отырып, сирек кездесетін *Linaria cretacea* өсімдік түрлерінің Қазақстанда және көршілес елдерде таралуы қарастырылған. Зерттеудің мақсаты — бұл түрдің Ақтөбе облысындағы таралу аймағын нақтылау, әсіресе борлы беткейлер оның мекендеу ортасына қолайлы жағдай туғызады. Гербарий деректерін талдау *Linaria cretacea*-ның шектеулі, бірақ айтарлықтай таралуын көрсетті, оның негізгі табылған жерлері Қазақстанда, сондай-ақ Ресей мен Украинада. Жұмыста осы түрдің өсуі үшін бор экожүйелерінің маңыздылығы атап көрсетіліп, оның таралуын жеңілдететін қоршаған орта жағдайлары сипатталған. Далалық зерттеулерде мекендеу орындары анықталды, бұл тұрақты мониторинг және популяцияның жай-күйі туралы мәліметтерді жаңарту қажеттілігін көрсетеді. Сондай-ақ мақалада *Linaria cretacea*-ның антропогендік әсерлерге және климаттың өзгеруіне әсері қарастырылып, сирек кездесетін өсімдіктер түрлерін қорғау шараларын әзірлеу қажеттілігіне баса назар аударылады. Зерттеу нәтижелері болашақ зерттеулер үшін жаңа мүмкіндіктер ашады, соның ішінде *Linaria cretacea*-ның генетикалық әртүрлілігін және басқа түрлермен өзара әрекеттесуін зерттеу, бұл сирек кездесетін түрді сақтау және қалпына келтіру стратегияларын тиімдірек дамытуға мүмкіндік береді.

Кілт сөздер: Linaria cretacea, таралуы, Қазақстан, электронды гербарий, далалық зерттеу, осалдық, мониторинг, өсімдіктерді қорғау.

М. Бакытжанова

Изучение распространения *Linaria cretacea* в Казахстане и сопредельных странах: данные электронного гербария

В данной статье рассматривается распространение редкого вида растений *Linariac retacea* в Казахстане и сопредельных странах с акцентом на данные электронного гербария. Целью исследования является уточнение ареала этого вида, особенно в Актюбинской области, где меловые склоны создают благоприятные условия для его обитания. Анализ данных гербария показал, что *Linaria cretacea* имеет ограниченное, но значительное распространение — основные находки приходятся на территорию Казахстана, а также России и Украины. В работе подчеркивается важность меловых экосистем для произрастания данного вида и описываются экологические условия, способствующие его распространению. В результате полевых исследований были выявлены местообитания, что свидетельствует о необходимости регулярного мониторинга и обновления данных о состоянии популяций. В работе также уделяется внимание уязвимости *Linaria cretacea* к антропогенным воздействиям и изменению климата, подчеркивается необходимость разработки мер по охране редких видов растений. Результаты ис-

следования открывают новые направления для будущих работ, в том числе по изучению генетического разнообразия и взаимодействий *Linaria cretacea* с другими видами, что позволит более эффективно разрабатывать стратегии сохранения и восстановления этого редкого вида.

Ключевые слова: *Linaria cretacea*, распространение, Казахстан, электронный гербарий, полевые исследования, уязвимость, мониторинг, охрана растений.

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Genetic diversity of the Kazakh Tobet dog and comparison with free-ranging dog populations

The Kazakh Tobet is a traditional livestock guardian dog (LGD) breed in Kazakhstan. A comparison of genetic diversity between the traditional breed and free-ranging (outbred) dogs makes it possible to better understand whether the genetic diversity of this breed is more similar to that of a structured breed population or an unstructured, free-ranging dog population. The aim of this study was therefore to assess the genetic diversity of the Kazakh Tobet and compare it with the genetic diversity of free-ranging dogs. A total of 107 Tobet samples from three regions of Kazakhstan and Mongolia and 55 free-ranging dogs were genotyped using 18 polymorphic microsatellite loci. The main parameters of genetic diversity — including mean number of alleles (Na), effective alleles (Ne), observed (Ho) and expected heterozygosity (He) and fixation index (F) — were evaluated. Tobet dogs showed a high level of genetic diversity (Na = 10.722, Ho = 0.781, He = 0.805 for the total populations), comparable to the values of outbred dogs (Na = 9.556, Ho = 0.776, He = 0.791). All four Tobet populations showed signs of internal diversity. Fixation index values were low or negative in most populations, suggesting that there is no strong inbreeding.

These results confirm the position of the Kazakh Tobet as a genetically rich and structurally complex LGD breed that is maintained without strict reproductive isolation. They also illustrate a paradox in the conservation of the Kazakh Tobet: while the high genetic diversity and admixture reflect the breed's adaptive success and functional selection history, formal recognition of the breed and long-term conservation require a strategic framework. In the case of the Kazakh Tobet, this does not mean imposing rigid reproductive isolation, but rather implementing a scientifically guided, open breeding system — that supports genetic monitoring, preserves functional traits, and protects against both genetic erosion and uncontrolled hybridization.

Keywords: Genetic profile, gene pool, genetic diversity, inbreeding, microsatellite marker, population genetics, Tobet breed.

Introduction

The Kazakh Tobet is one of the oldest and culturally most important guard dog breeds in Kazakhstan. Historically used by Kazakh nomad shepherds to guard livestock during seasonal migrations and was primarily selected for behavioral and functional traits such as alertness, endurance, weather resistance and independence. Despite its cultural importance, the breed remained largely uncharacterized at the genomic level until recently. Fragmented breeding practices, lack of centralized registration and increased crossbreeding with local or import breeds have raised concerns about the preservation of the genetic identity and functional capacity of the Kazakh Tobet.

To assess the current genetic status of the Kazakh Tobet, comparisons with free-ranging (or outbred) dog populations are an important benchmark. Free-ranging dogs, which reproduce without pedigree control or artificial selection, generally show a high degree of heterozygosity and allelic richness. These parameters make them a meaningful reference point for assessing whether a traditional breed such as the Tobet exhibits patterns of diversity consistent with purebred breeds, or whether it resembles the broader, genetically variable population of free-ranging dogs.

In parallel, several studies have begun in recent years to investigate the genetic characterization of working breeds. It has been shown that many of these breeds frequently interbreed with local free-ranging dogs [1, 2]. This has led to a paradigm shift: instead of interpreting genetic admixture as a threat to breed purity, it is increasingly seen as a mechanism that improves adaptability and preserves working traits, espe-

cially under extensive pastoral conditions. However, the Kazakh Tobet remains underrepresented in these discussions and its genetic diversity compared to outbred dog populations has not been systematically studied.

In this study, we present a comprehensive assessment of the genetic diversity and structure of the Kazakh Tobet in comparison to free-ranging dog populations. Using 18 polymorphic microsatellite markers, we analyzed samples of Kazakh Tobet from three regions of Kazakhstan and Mongolia. These were compared with a reference population of outbred dogs. The main genetic parameters, including observed and expected heterozygosity, average number of alleles, effective allele number and fixation index were evaluated. The main objective of this work is to quantify the genetic diversity of the Kazakh Tobet, determine its relationship to the outbred dog gene pool, and provide an empirical basis for strategies to preserve the breed.

Materials and methods of the research

Objects of the research

The research protocol was reviewed and approved by the Bioethics Committee of the RSE at REM Institute of Molecular Biology and Biochemistry named after M.A. Aitkhozhin, under the Committee of Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Protocol No. 1, August 18, 2023). The study was conducted in accordance with the "Bioethical Rules for Conducting Research Involving Humans and Animals", the legislation of the Republic of Kazakhstan, and the principles of the European Convention on Bioethics. Importantly, no invasive experiments were performed on animals during the study. All research procedures involved the collection of biological materials from dogs through minimally invasive methods, posing no harm or distress to the animals.

Biological samples were obtained from Kazakh Tobet dogs during field expeditions, exhibitions, and specialized breed-related events. Evaluation of each dog's conformity to the Kazakh Tobet breed standard was conducted by certified cynologists from the national organization "KANSONAR". These experts met the qualification requirements and had extensive experience with both national Kazakh breeds and the broader Central Asian Shepherd Dog group, to which the Kazakh Tobet is classified. The assessments were based on the official breed standard for the Kazakh Tobet, approved by the Decree of the Ministry of Ecology and Natural Resources of the Republic of Kazakhstan No. 101 dated March 30, 2023. For comparative purposes, a control group of free-breeding (outbred) dogs was included. These dogs, known for their high genetic variability and adaptive potential, were sampled from the animal welfare organizations "Tailed Paradise" and "New Chance".

Two types of biological material were collected from Kazakh Tobet and outbred dogs:

- buccal swabs were obtained by gently brushing the inner cheek surface. Samples were placed in sterile tubes containing phosphate-buffered saline (PBS). This non-invasive and painless procedure was used for both Kazakh Tobet and outbred dogs.

- peripheral blood samples (up to 50 ml) were collected from the leg vein using EDTA-coated vacuum tubes by a licensed veterinarian experienced in research sampling. All procedures were carried out under sterile conditions and were minimally invasive.

All samples were promptly transported in a portable refrigerated container to the Institute of Genetics and Physiology. Upon arrival, samples were stored at −80 °C until further molecular genetic analyses were conducted.

Additionally, each sampled dog was photographed, and owners were asked to complete a detailed questionnaire. The questionnaire gathered data on the dog's age, sex, origin, current place of residence, physical description, and measurements. All data were digitized and entered into an electronic database. Informed consent was obtained from each dog's owner prior to the collection of samples and genetic testing.

Methods of the research

DNA extraction

DNA was extracted using "QIAamp Fast DNA Tissue Kit" (Qiagen, Germany) kits according to the manufacturer's protocol. The qualitative and quantitative characteristics of the isolated DNA were determined using Qubit4.0 (Invitrogen, USA) or 2100 Expert (Agilent Technologies, USA).

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Microsatellite analysis

Microsatellite analysis was performed with the SeqStudio™ Genetic Analyser (Thermo Fisher Scientific, USA) using the Thermo Scientific Canine Genotypes Panel 1 (Thermo Fisher Scientific, USA), which contains 19 loci recommended by ISAG for dog (AHTk211, CXX279, REN169O18, INU055, REN54P11, INRA21, AHT137, REN169D01, AHTh260, AHTk253, INU00 5, INU030.

Methods for bioinformatics and statistical processing of microsatellite analysis data

Genetic evaluation based on allele frequencies was performed using the programs GenAlEx 6.5 [3] and Cervus [4]. We evaluated indicators such as the average (Na) and effective (Ne) number of alleles, the observed (Ho) and expected (He) heterozygosity, and the agreement with the Hardy-Weinberg distribution.

Results

Genomic DNA was extracted from all collected biological samples of Kazakh Tobet dogs (n = 163), and its quality and quantity were assessed using standard protocols. Likewise, DNA was isolated from biomaterials collected from outbred dogs (n = 55), followed by evaluation of DNA integrity and concentration.

Microsatellite genotyping was conducted for 18 autosomal loci in 107 Kazakh Tobet dogs representing four geographic populations: South Kazakhstan (n = 73; hereafter Pop 1), East Kazakhstan (n = 16; Pop 2), North Kazakhstan (n = 8; Pop 3), and Bayan-Ölgii, Mongolia (n = 4; Pop 4). Additionally, 55 outbred dogs were genotyped as a comparative group. Allele frequencies were calculated for each locus, and key parameters of genetic variability were assessed for both Kazakh Tobet and outbred dogs (Tables 1, 2).

Table 1

Genetic variability of Kazakh Tobet dogs

Population	Locus	Na	Ne	Ho	He	F
Pop1	AHTk211	6,000	3,628	0,616	0,724	0,149
	CXX0279	10,000	5,613	0,792	0,822	0,037
	REN169O18	11,000	5,721	0,808	0,825	0,021
	INU055	9,000	4,992	0,849	0,800	-0,062
	REN54P11	10,000	5,709	0,877	0,825	-0,063
	INRA21	8,000	5,557	0,918	0,820	-0,119
	AHT137	14,000	6,337	0,904	0,842	-0,074
	REN169D01	11,000	6,307	0,932	0,841	-0,107
	AHTh260	12,000	4,329	0,712	0,769	0,074
	AHTk253	9,000	2,791	0,466	0,642	0,274
	INU005	10,000	3,335	0,603	0,700	0,139
	INU030	7,000	4,315	0,781	0,768	-0,016
	FH2848	9,000	5,789	0,877	0,827	-0,060
	AHT121	12,000	7,860	0,795	0,873	0,090
	FH2054	12,000	6,563	0,767	0,848	0,095
	REN162C04	13,000	6,149	0,833	0,837	0,005
	AHTh171	12,000	8,167	0,877	0,878	0,001
	REN247M23	9,000	4,372	0,685	0,771	0,112
	Mean	10,222	5,418	0,783	0,801	0,027
	SE	0,495	0,341	0,029	0,015	0,024
Pop2	AHTk211	5,000	4,303	0,813	0,768	-0,059
	CXX0279	6,000	3,657	0,813	0,727	-0,118
	REN169O18	6,000	4,697	0,875	0,787	-0,112
	INU055	7,000	5,389	0,875	0,814	-0,074
	REN54P11	7,000	4,031	0,938	0,752	-0,247
	INRA21	6,000	3,969	0,625	0,748	0,164
	AHT137	9,000	7,314	0,938	0,863	-0,086
	REN169D01	8,000	5,069	0,875	0,803	-0,090
	AHTh260	7,000	4,303	0,688	0,768	0,104
	AHTk253	7,000	3,737	0,750	0,732	-0,024
	INU005	7,000	4,096	0,625	0,756	0,173

Continuation of Table 1

Population	Locus	Na	Ne	Ho	He	F
Pop2	INU030	5,000	4,197	0,625	0,762	0,179
	FH2848	6,000	4,452	0,813	0,775	-0,048
	AHT121	10,000	7,014	0,938	0,857	-0,093
	FH2054	9,000	5,333	0,813	0,813	0,000
	REN162C04	8,000	4,531	0,750	0,779	0,038
	AHTh171	8,000	3,580	0,750	0,721	-0,041
	REN247M23	5,000	2,926	0,625	0,658	0,050
	Mean	7,000	4,589	0,785	0,771	-0,016
	SE	0,343	0,264	0,026	0,012	0,027
Pop3	AHTk211	4,000	3,048	0,375	0,672	0,442
	CXX0279	6,000	5,120	0,875	0,805	-0,087
	REN169O18	5,000	3,556	0,875	0,719	-0,217
	INU055	5,000	3,879	0,750	0,742	-0,011
	REN54P11	6,000	4,414	0,750	0,773	0,030
	INRA21	6,000	4,741	1,000	0,789	-0,267
	AHT137	6,000	4,923	1,000	0,797	-0,255
	REN169D01	8,000	5,818	0,875	0,828	-0,057
	AHTh260	8,000	6,400	0,750	0,844	0,111
	AHTk253	8,000	6,400	0,875	0,844	-0,037
	INU005	6,000	3,200	0,625	0,688	0,091
	INU030	6,000	4,414	0,875	0,773	-0,131
	FH2848	5,000	4,741	0,750	0,789	0,050
	AHT121	7,000	5,333	0,750	0,813	0,077
	FH2054	6,000	5,120	0,875	0,805	-0,087
	REN162C04	6,000	4,267	0,625	0,766	0,184
	AHTh171	6,000	4,129	0,625	0,758	0,175
	REN247M23	6,000	3,765	0,500	0,734	0,319
	Mean	6,111	4,626	0,764	0,774	0,018
	SE	0,254	0,230	0,039	0,012	0,045
Pop4	AHTk211	5,000	3,704	0,900	0,730	-0,233
	CXX0279	7,000	2,941	0,900	0,660	-0,364
	REN169O18	5,000	4,082	0,900	0,755	-0,192
	INU055	6,000	4,000	0,600	0,750	0,200
	REN54P11	5,000	3,448	0,500	0,710	0,296
	INRA21	6,000	5,000	0,800	0,800	0,000
	AHT137	9,000	6,452	0,900	0,845	-0,065
	REN169D01	8,000	5,714	0,900	0,825	-0,091
	AHTh260	6,000	3,704	0,900	0,730	-0,233
	AHTk253	3,000	1,504	0,200	0,335	0,403
	INU005	4,000	2,667	0,700	0,625	-0,120
	INU030	5,000	4,167	0,900	0,760	-0,184
	FH2848	7,000	4,762	0,900	0,790	-0,139
	AHT121	7,000	5,405	0,800	0,815	0,018
	FH2054	7,000	4,000	0,300	0,750	0,600
	REN162C04	6,000	4,348	0,900	0,770	-0,169
	AHTh171	6,000	3,571	1,000	0,720	-0,389
	REN247M23	6,000	5,128	0,900	0,805	-0,118
	Mean	6,000	4,144	0,772	0,732	-0,043
	SE	0,333	0,276	0,054	0,027	0,062
All (n=107)	AHTk211	6,000	3,813	0,654	0,738	0,113
	CXX0279	11,000	5,335	0,811	0,813	0,002
	REN169O18	11,000	5,632	0,832	0,822	-0,011

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Continuation of Table 1

Population	Locus	Na	Ne	Ho	He	F
All (n=107)	INU055	10,000	5,110	0,822	0,804	-0,023
	REN54P11	10,000	5,516	0,841	0,819	-0,027
	INRA21	8,000	5,408	0,869	0,815	-0,066
	AHT137	14,000	7,198	0,916	0,861	-0,064
	REN169D01	11,000	6,439	0,916	0,845	-0,084
	AHTh260	13,000	4,733	0,729	0,789	0,076
	AHTk253	11,000	3,085	0,514	0,676	0,239
	INU005	10,000	3,433	0,617	0,709	0,130
	INU030	7,000	4,504	0,776	0,778	0,003
	FH2848	9,000	5,943	0,860	0,832	-0,034
	AHT121	12,000	8,255	0,813	0,879	0,075
	FH2054	13,000	6,584	0,738	0,848	0,129
	REN162C04	15,000	6,293	0,811	0,841	0,035
	AHTh171	12,000	6,895	0,850	0,855	0,005
	REN247M23	10,000	4,233	0,682	0,764	0,107
	Mean	10,722	5,467	0,781	0,805	0,034
	SE	0,547	0,321	0,025	0,013	0,020

Table 2

Genetic variability of outbred dogs

Pop	Locus	Na	Ne	Ho	He	F
Outbred (n=55)	AHTk211	6,000	4,569	0,764	0,781	0,022
	CXX0279	8,000	5,004	0,727	0,800	0,091
	REN169O18	10,000	5,490	0,855	0,818	-0,045
	INU055	9,000	4,549	0,636	0,780	0,184
	REN54P11	10,000	5,879	0,818	0,830	0,014
	INRA21	7,000	4,632	0,818	0,784	-0,043
	AHT137	12,000	5,996	0,891	0,833	-0,069
	REN169D01	11,000	5,955	0,891	0,832	-0,071
	AHTh260	12,000	5,891	0,873	0,830	-0,051
	AHTk253	7,000	3,085	0,709	0,676	-0,049
	INU005	12,000	3,658	0,636	0,727	0,124
	INU030	6,000	3,168	0,709	0,684	-0,036
	FH2848	8,000	6,044	0,727	0,835	0,129
	AHT121	13,000	8,509	0,891	0,882	-0,010
	FH2054	11,000	5,762	0,764	0,826	0,076
	REN162C04	12,000	4,844	0,873	0,794	-0,100
	AHTh171	11,000	5,123	0,764	0,805	0,051
	REN247M23	7,000	3,555	0,618	0,719	0,140
	Mean	9,556	5,095	0,776	0,791	0,020
	SE	0,550	0,308	0,022	0,013	0,020

The percentage of polymorphic loci in the sample of Kazakh Tobet dogs was 100 % and a total of 193 alleles were identified. Pop 1 had the highest average number of alleles per locus (Na=10.222±0.495). In comparison, Pop 2 and Pop 3 showed moderate genetic diversity, with mean Na values of 7.000±0.343 and 6.111±0.254, respectively. The lowest genetic diversity among the four populations was observed in Pop 4, where the mean Na value was 6.000±0.333. The highest number of alleles was found at loci REN162C04, AHT137, AHTh260, FH2054, AHT121 and AHTh171, each with 12 to 15 alleles. The lowest number of alleles was found for the AHTk211 locus with 6 alleles. The average number of effective alleles for the all samples analyzed was 5.467±0.321 and ranged from 4.144±0.276 in Pop 4 to 5.418±0.341 in Pop 1. The highest observed heterozygosity was found in Pop 2 (Ho=0.785±0.026), followed by Pop 1 (Ho=0.783±0.029) and Pop 3 (Ho=0.764±0.039). The highest expected heterozygosity was found in Pop 1 (He=0.801±0.015), while Pop 3 (He=0.774±0.012) and Pop 2 (He=0.771±0.012) also showed significant but

slightly lower heterozygosity. In contrast, Pop 4 had the lowest heterozygosity values, with Ho at 0.772±0.054 and He at 0.732±0.027. The fixation index F was negative in Pop 2 (-0.016±0.027) and Pop 4 (-0.043±0.062), indicating an excess of heterozygotes in these populations. In contrast, Pop 1 (0.027±0.024) and Pop 3 (0.018±0.045) had positive F-values, indicating a slight lack of heterozygotes.

The percentage of polymorphic loci in the sample of outbred dogs was 100 %, a total of 172 alleles were identified. The average number of alleles per locus (Na) was 9.556±0.550. The highest genetic diversity among the loci was found for the AHT121 locus, which had 13 alleles. The INU030 locus had the lowest number of alleles — only 6. The average Ne value was 5.095±0.308 and varied from 3.085 for the AHTk253 locus to 8.509 for the AHT121 locus. The average observed heterozygosity (Ho) for all analyzed loci was 0.776±0.022, which is close to the average expected heterozygosity (He), which was 0.791±0.013. The highest observed heterozygosity was recorded for the AHT137, REN169D01 and AHT121 loci, where Ho reached 0.891. The lowest observed heterozygosity was at the REN247M23 locus (Ho=0.618). The fixation index was generally close to zero (F=0.020±0.020), indicating that there is neither a significant lack nor excess of heterozygotes in the population. Negative F-values, indicating an excess of heterozygotes, were found for REN169O18 (-0.045), AHT137 (-0.069), REN169D01 (-0.071) and other loci. At the same time, positive F-values, indicating a slight heterozygote deficiency, were found at the loci INU005 (0.124), FH2848 (0.129) and REN247M23 (0.140).

HWE assessment in the analyzed sample of Kazakh Tobet dogs showed a deviation from HWE for seven loci (INRA21, AHT137, AHTh260, AHTk253, FH2054, REN162C04 and AHTh171 at P<0.0011) and in the analyzed sample of outbred dogs — for four loci (CXX0279 at P<0.05, INU055 at P<0.05, FH2054 and INU005 at P<0.001).

A comprehensive analysis of the genetic parameters of outbred and Kazakh Tobet dogs (Fig.) revealed relatively similar values for all parameters assessed. At the same time, the Kazakh Tobet population even showed a slightly higher genetic diversity compared to the outbred dogs, despite a relatively small deficit of heterozygotes: the F-fixation index was higher in the Kazakh Tobets (0.034) than in the outbred dogs (0.02).

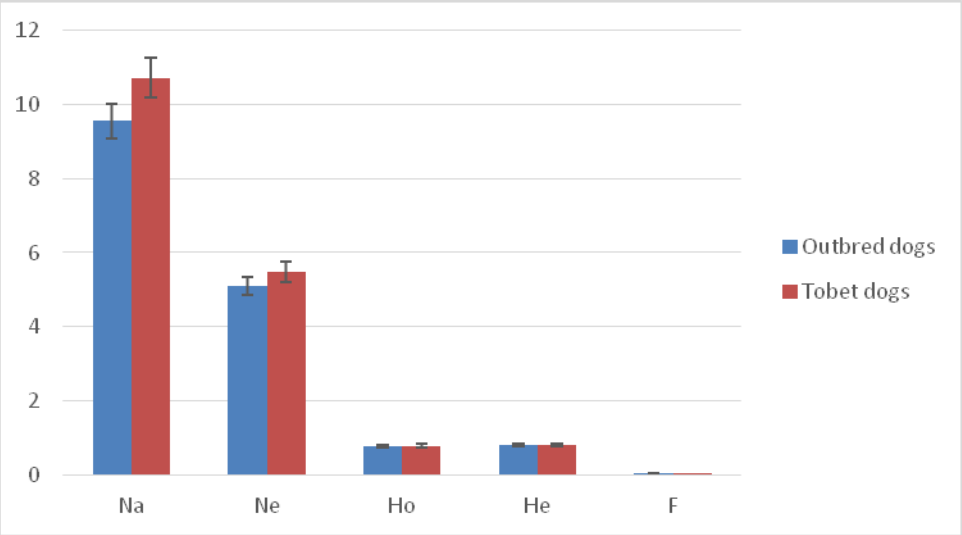

Figure. Comparative analysis of genetic diversity parameters of Kazakh Tobet and outbred dogs

Discussion

This study provides the population genetic analysis of the Kazakh Tobet, a traditional LGD breed, based on 18 highly polymorphic microsatellite loci. By analyzing 107 Kazakh Tobet dogs from three regions of Kazakhstan and Mongolia, and comparing them with 55 free-breeding dogs, we assessed the genetic diversity that define the contemporary gene pool of this indigenous breed.

The Kazakh Tobet demonstrated high levels of genetic variability across all metrics analyzed. A total of 193 alleles were identified, and 100 % of loci were polymorphic. The average number of alleles per locus (Na) reached 10.722—substantially higher than values reported for many molossoid and non-molossoid breeds. For example, the Kazakh Tobet parameters were higher than those of the Tibetan Mastiff (Na=7.7,

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panel of 10 STR loci [5]), the in English Bulldog (Na=6.455 and Ne=2.722; panel of 33 STR loci [6], the French Bulldog (Na=5.1; Ne=2.9; panel of 18 STR loci [7]). In addition, the genetic analysis performed on the basis of a panel of 10 STR markers showed lower values of observed heterozygosity (Ho) compared to Kazakh Tobets for such breeds from the molossoid group as Boxers, Staffordshire Bull Terriers and Rottweilers (Ho = 0.51, 0.63 and 0.47, respectively) when analyzing a panel of 15 markers [8], for the Tibetan Mastiff and French Bulldog (Ho = 0.694-0.76, and 0.6077, respectively) when analyzing a panel of 10 markers [9]. However, similar values of over 70 % observed heterozygosity were also found for several non-molossoid dog breeds: for the Korean Dongyonggi (Ho = 0.7266) when analyzing 10 microsatellite loci [10], for the Italian Pointer and the Podenco (Ho = 0.723 and 0.710-0.718) when analyzing a similar panel of 19 microsatellite loci [11], for the Yorkshire Terrier (Ho= 0.73) when analyzing 15 STR markers [8].

In population genetic studies of dog breeds, high observed heterozygosity is often interpreted as an indication of recent admixture, large effective population size or lack of strict reproductive isolation. Indeed, our own control group of outbred dogs exhibited high heterozygosity (Ho = 0.776), very similar to that of the Kazakh Tobet. This supports the conclusion that the Kazakh Tobet dogs are still kept in an open mating system with varying degrees of reproductive isolation.

However, recent genomic studies have shown that LGD breeds worldwide often do not exhibit strict reproductive isolation and show extensive genetic overlap with outbred dogs due to their traditional role in rural and nomadic livestock systems. Dutrow et al. were the first to point out the genetic link between purebred and free-ranging dogs [1]. Coutinho-Lima et al. showed the widespread nature of this relationship within LGD breeds and suggested that reproductive isolation may not be necessary to maintain highly specialized dogs [2]. This has led to the growing consensus that reproductive isolation is not a prerequisite for the preservation of important working traits. Rather, it is cultural and functional selection—based on performance and behavior in the field—that maintains the integrity of LGD populations. In this context, the high levels of heterozygosity observed in Kazakh Tobets are not indicative of breed degradation, but reflect the adaptive diversity maintained by an open breeding system. Similar results have been reported for other traditional LGD breeds such as the Turkish Kangal [12] and the Portuguese Castro Laboreiro dog [13], where genomic analyses revealed an extensive exchange of alleles with local outbred populations. From this perspective, the high genetic diversity observed in Kazakh Tobets should not be seen as a threat to the conservation of the breed, but rather as a sign of resilience and adaptability—traits that are essential for their survival in the harsh steppe and mountainous landscapes of Central Asia.

Although the genetic diversity of the Kazakh Tobet breed was high overall, it was not evenly distributed across all populations. There are clear differences between the four populations studied. The southern population showed the highest genetic diversity, but a slight deficit of heterozygotes indicated low inbreeding (positive F-value). The eastern and northern populations showed moderate diversity, with the former showing an excess of heterozygotes (negative F-values), indicating inbreeding, and the latter showing a slight deficit of heterozygotes. The Mongolian population was characterized by the lowest genetic diversity and the strongest evidence of crossbreeding (the lowest negative F-value).

Taken together, these results position the Kazakh Tobet as a genetically rich and structurally complex LGD breed maintained under conditions of semi-natural selection and open gene flow. They also illustrate a paradox in the conservation of the Kazakh Tobet: while the high genetic diversity and admixture reflect the breed's adaptive success and functional selection history, formal recognition of the breed and long-term conservation require a strategic framework. In the case of the Kazakh Tobet, this does not mean imposing rigid reproductive isolation, but rather implementing a scientifically guided, open breeding system — that supports genetic monitoring, preserves functional traits, and protects against both genetic erosion and uncontrolled hybridization.

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Author Contributions

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Қазақ төбет иттерінің генетикалық әртүрлілігін бағалау және оны жабайы иттердің популяциясымен салыстыру

Қазақтың төбеті — Қазақстан аумағында малды қорғау қызметін орындаған дәстүрлі ит тұқымы. Текті тұқымды топтар мен жабайы иттердің популяциялары арасындағы генетикалық әртүрлілігін салыстырмалы талдау тұқымның генофондының құрылымдық популяция белгілеріне сәйкес келетіндігін немесе ұйымдастырылмаған, генетикалық жағынан әр түрлі топтардың сипаттамаларын сақтайтындығын анықтауға мүмкіндік береді. Бұл зерттеу жұмысының негізгі мақсаты — Қазақ төбетінің генетикалық әртүрлілік деңгейін бағалауға және оны жабайы иттердің көрсеткіштерімен салыстыруға бағытталған. Жұмыс аясында Қазақстанның үш өңірінен және Монғолиядан іріктелген төбет тұқымды иттердің 107 түрі, сондай-ақ жабайы иттердің 55 түрі талданды. Генотиптеу 18 полиморфты микросателлиттік локустар бойынша жүргізілді. Генетикалық вариацияның негізгі

параметрлері есептелді: аллельдердің орташа саны (Na), аллельдердің тиімді саны (Ne), бақыланатын (Ho) және күтілетін гетерозиготалық (He) және фиксация индексі (F). Нәтижесінде жабайы иттермен салыстырғанда (Na = 9.556, Ho = 0.776, He = 0.791) төбет генетикалық әртүрліліктің жоғары деңгейіне ие екенін көрсетті (Na = 10.722, Ho = 0.781, He = 0.805). Зерттеуге қатысқан төбеттің барлық төрт популяциясында жоғары гетерогенділік тіркелді. Бекіту индексінің теріс немесе нөлге жақын мәндері көп жағдайда айқын инбридингтің жоқтығын көрсетеді. Ұсынылған нәтижелер Қазақ төбетінің қатаң репродуктивті оқшаулау болмаған жағдайда қалыптасқан генетикалық әр түрлі және ішкі сараланған тұқым екенін көрсетеді. Мұндай ерекшеліктер тау жыныстарын сақтау мәселелерінде белгілі бір парадокс тудырады: жоғары өзгергіштік және қоспа белгілерінің болуы оның бейімделгіш икемділігі мен функционалдық тұрақтылығын көрсетеді, бірақ сонымен бірге тану мен ұзақ мерзімді сақтаудың стратегиялық тәсілін қажет етеді. Төбет иттерінің жағдайында қатаң репродуктивті оқшаулауды енгізбей, тұрақты генетикалық бақылауды және негізгі тұқымдық белгілерді сақтауды, сонымен қатар генетикалық деградациядан да, бақылаусыз будандастырудан да қорғауды көздейтін ашық тұқымды өсірудің ғылыми негізделген үлгісін жасау дұрыс шешім деп санаймыз.

Кілт сөздер: генетикалық профиль, генофонд, генетикалық әртүрлілік, инбридинг, микросателлиттік маркер, популяциялық генетика, төбет тұқымы.

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Оценка генетического разнообразия собак породы Казахский Тобет и её сравнение с популяциями беспородных собак

Казахский Тобет представляет собой традиционную породу собак, исторически выполнявшую функцию охраны скота на территории Казахстана. Сравнительный анализ генетического разнообразия породных и беспородных собак позволяет установить, соответствует ли генофонд породы признакам структурированной популяции или сохраняет характеристики неорганизованных, генетически разнородных групп. Настоящее исследование было направлено на оценку уровня генетического разнообразия Казахского Тобета и его сопоставление с показателями беспородных собак. В рамках работы были проанализированы 107 образцов собак породы Тобет, отобранных в трёх регионах Казахстана и Монголии, а также 55 образцов беспородных собак. Генотипирование осуществлялось по 18 полиморфным микросателлитным локусам. Были рассчитаны ключевые параметры генетической изменчивости: среднее количество аллелей (Na), эффективное число аллелей (Ne), наблюдаемая (Ho) и ожидаемая гетерозиготность (He), а также индекс фиксации (F). Результаты показали, что Тобет обладает высоким уровнем генетического разнообразия (Na = 10.722, Ho = 0.781, He = 0.805), сравнимым с показателями беспородных собак (Na = 9.556, Ho = 0.776, He = 0.791). Высокая гетерогенность была зафиксирована во всех четырёх популяциях Тобета, участвовавших в исследовании. Отрицательные или близкие к нулю значения индекса фиксации в большинстве случаев свидетельствуют об отсутствии выраженного инбридинга. Представленные результаты свидетельствуют о том, что Казахский Тобет является генетически разнообразной и внутренне дифференцированной породой, формировавшейся в условиях отсутствия строгой репродуктивной изоляции. Такие особенности создают определённый парадокс в вопросах сохранения породы: высокая изменчивость и наличие признаков примеси отражают её адаптивную пластичность и функциональную устойчивость, но одновременно требуют более стратегического подхода к признанию и долгосрочному сохранению. В случае Тобета целесообразным представляется не введение строгой репродуктивной изоляции, а разработка научно обоснованной модели открытого разведения, предусматривающей регулярный генетический мониторинг, сохранение ключевых породных признаков и защиту как от генетической деградации, так и от неконтролируемой гибридизации.

Ключевые слова: генетический профиль, генофонд, генетическое разнообразие, инбридинг, микросателлитный маркер, популяционная генетика, порода Тобет.

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Pharmaceutical development and standardization of *Salvia stepposa* tablets

In response to the growing threat of antimicrobial resistance, plant-derived compounds are increasingly being explored as safe and effective alternatives to conventional antibiotics. This study focused on the development of solid oral dosage forms based on a dry extract of *Salvia stepposa* (MVSHS-40), rich in phenolic acids and flavonoids with proven antimicrobial and anti-inflammatory activity. The extract was obtained using microwave-assisted extraction (MAE) with 40 % ethanol, yielding up to 6 % rosmarinic acid as the dominant bioactive compound, along with caffeic and p-coumaric acids, and flavonoids such as apigenin, naringenin, and luteolin. The tablets were formulated by direct compression using microcrystalline cellulose, EMDEX, citric acid, and calcium stearate, with some variants incorporating essential oils. Out of more than 30 experimental variants, five optimized compositions were selected. Physicochemical and pharmacopeial evaluations confirmed excellent disintegration times (<12 minutes), dissolution rates (>79 %), uniform mass, high mechanical strength (≥107 N), and minimal friability (≥99.99 %). HPLC-UV/MS analysis quantified 3.046 mg of rosmarinic acid per tablet. Comparative analysis revealed a 5.5-fold increase in active content over commercial *Salvia officinalis* tablets while avoiding thujone-related safety concerns. Stability studies over 18 months demonstrated sustained integrity of pharmacological properties. The resulting MVSHS-40 tablets meet all quality specifications and represent a safe, stable, and effective herbal formulation with potential applications in the prevention and treatment of upper respiratory tract infections.

Keywords: *Salvia stepposa*, microwave-assisted extraction, rosmarinic acid, phenolic compounds, HPLC-MS/MS, phytopharmaceuticals.

Introduction

Amid the growing problem of microbial antibiotic resistance, there is an urgent need to find new, effective, and safe medicinal agents. Plant-derived bioactive compounds are attracting increasing attention due to their low toxicity and ability to inhibit the growth of pathogenic and opportunistic microorganisms [1]. In particular, herbal extracts have become a promising source of natural antimicrobial agents with a broad spectrum of activity and high bioavailability. Steppe sage (*Salvia stepposa*) is a plant known for its high content of phenolic compounds and flavonoids, which exhibit pronounced antimicrobial activity [2]. In recent studies, thin-layer chromatography (TLC) and high-performance liquid chromatography coupled with mass spectrometric detection (HPLC-MS/MS) were used to identify key components of sage extracts, among which rosmarinic acid was dominant [3], accounting for up to 6 % of the extract mass. Additionally, the extracts contained caffeic and p-coumaric acids, as well as flavonoids such as naringenin, apigenin, and luteolin. The antimicrobial activity of these extracts was confirmed through testing on bacterial strains (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa*) and fungi of the genus *Candida albicans* [4]. The results showed significant inhibition of microbial growth, indicating the potential use of steppe sage extracts as natural antimicrobial agents.

Solid oral dosage forms, particularly tablets, dominate modern pharmaceutical production due to their versatility, cost-efficiency, and ease of administration [5]. Among them, tablets provide a targeted delivery system for the treatment of upper respiratory tract infections. The goal of this research was to develop a tablet formulation based on a dry extract of *Salvia stepposa* (MVSHS-40), recognized for its anti-inflammatory and antibacterial activity.

Experimental

Plant material. The wild species *Salvia stepposa* Des. -Schost (syn. *Salvia dumetorum*), native to the flora of Kazakhstan, was collected during field expeditions in the Karaganda region (Republic of Kazakhstan) at coordinates N 49°88898’; E 73°15569’, during its budding and flowering stages in July–August 2024.

Microwave-assisted extraction (MAE). MAE of *Salvia stepposa* Des.-Schost leaves was performed according to the methods described in the literature [6]. For MAE of *Salvia stepposa*, a 40 % hydroalcoholic

solution was used as the extracting solvent. Air-dried *Salvia* leaves weighing 20 g were placed into a 500 ml round-bottom flask, and 200 ml of the solvent was added, maintaining a solid-to-liquid ratio of 1:10 (mw%: vw%). The flask containing the material was placed into a domestic microwave oven operating at a frequency of 2.45 GHz and connected to a reflux condenser to condense vapors. Extraction was performed four times until a nearly clear solution was obtained, with microwave irradiation lasting 4 minutes at a power of 300 W. The exposure was carried out in 10-second cycles with 1-minute intervals to prevent overheating and boiling of the solvent. The temperature of the mixture was monitored every minute using an IR-T1 CONDTROL infrared thermometer. The obtained extracts were concentrated under vacuum on a Labtech IR-1LT rotary evaporator to preserve the bioactive compounds without degradation. Subsequently, the dry extract was obtained by vacuum drying at temperatures not exceeding 40 °C, ensuring the preservation of thermolabile components and producing a stable powder suitable for further use in pharmaceutical formulations.

High-Performance Liquid Chromatography. The analysis of rosmarinic acid in the tablets was performed using high-performance liquid chromatography (HPLC) combined with ultraviolet (UV) detection and real-time tandem mass spectrometry (ESI-MS/MS) according to the methodology [7]. The analysis was carried out on an Agilent 1260 Infinity HPLC system (Agilent Technologies, USA) equipped with a G1311C 1260 Pump VL (four-channel pump), G1329B 1260 ALS autosampler, G1316A 1260 TCC column thermostat, G1314C 1260 VWD VL+ variable wavelength detector, and a G6130A Quadrupole LC-MS/MS mass spectrometer. The system was operated using ChemStation software under Windows NT.

Chromatographic separation was performed on a reversed-phase Zorbax Eclipse Plus C18 column (150 mm × 4.6 mm, 3.5 μm, Agilent Technologies, USA). A gradient elution system was used with mobile phase A (2.5 % formic acid in water) and mobile phase B (2.5 % formic acid in acetonitrile). The gradient profile was as follows: 0.00 min—3 % B, 7.00 min—20 % B, 7.10 min—30 % B, 27.00 min—40 % B, 35.00 min—50 % B, 35.10 min—20 % B, 40.00 min—3 % B. The flow rate was 0.4 mL/min, and the column temperature was maintained at 30 °C. Tablets and standard of rosmarinic acid were dissolved in a 1:1 (v/v) mixture of acetonitrile and purified water. The injection volume was 20 μL for tablet and standard solutions. The eluent passed through the UV detector before reaching the mass spectrometry interface. UV detection wavelengths were set at 280 nm and 360 nm.

Mass spectrometric detection with electrospray ionization was performed in negative mode with the following optimized parameters: capillary temperature 350 °C; drying gas N_2 at 8 L/min; nebulizer pressure 45 psi. Data collection was carried out using the multiple reaction monitoring (MRM) method, which tracks specific mass transitions during the corresponding retention time windows.

Quantitative determination of rosmarinic acid was performed using the Agilent 1260 Infinity HPLC system.

The percentage of rosmarinic acid (X) in tablets containing dry extract of *Salvia stepposa* leaves was calculated using the formula:

$$X = \frac{S_1 \cdot m_0 \cdot 1 \cdot P \cdot 100}{S_0 \cdot m_1 \cdot 1.100}, \quad (1)$$

where:

S_1 — peak area of rosmarinic acid;

m_0 — mass of the standard sample of rosmarinic acid, g;

m_1 — mass of tablets containing dry extract of *Salvia stepposa* leaves, g;

P — content of rosmarinic acid in the standard sample, expressed as a percentage. Rosmarinic acid (molecular formula $C_{18}H_{16}O_8$), CAS — 20283-92-5, purity 98 % (Sigma-Aldrich, USA).

Methods of standardization of tablets [8, 9].

Description. According to the State Pharmacopoeia of the Republic of Kazakhstan (SPRK), Vol. 1, p. 550, general monograph "Tablets" and Pharmacopoeia of the Eurasian Economic Union (EAEU) 2.1.6.0.

Average tablet mass. The average mass of tablets was determined in accordance with SPRK Vol. I, 2.9.5 and EAEU Pharmacopoeia 2.1.9.5.

Disintegration. The disintegration test was performed according to SPRK Vol. I, 2.9.1 and EAEU Pharmacopoeia 2.1.9.1 using a Julabo disintegration tester (Germany).

Dissolution. The dissolution test was carried out in accordance with SPRK Vol. I, 2.9.3 and EAEU Pharmacopoeia 2.1.9.3.

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Friability. Tablet friability was evaluated according to SPRK Vol. I, 2.9.7 and EAEU Pharmacopoeia 2.1.9.6 using an AE-1 friability tester (Charles Isch AG).

Tablet crushing resistance. The crushing resistance was determined according to SPRK Vol. I, 2.9.8 and EAEU Pharmacopoeia 2.1.9.7 using an HC 6.2 hardness tester (Kraemer Elektronik GmbH).

Microbiological purity. Testing was carried out according to SP RK Vol. I, 2.6.12, SPRK Vol. I, 2.6.13, SPRK Vol. I, 5.1.4 (category 3B), and EAEU Pharmacopoeia 2.3.1.4.

Loss on drying. Determination of loss on drying was performed according to SPRK Vol. I, 2.2.32 "Loss on Drying" and EAEU Pharmacopoeia 2.1.2.31.

Results and Discussion

Excipients play a vital role in tablet formulation, influencing tablet integrity, disintegration, drug release, and stability. Moisture content was tightly controlled to improve flowability and compressibility.

Five optimal compositions were identified from over 30 experimental variants (Table 1). MVSHS-40 served as the active pharmaceutical ingredient (API). Excipients included microcrystalline cellulose (MCC 105) as a binder and disintegrant, EMDEX (dextrates) as a filler, citric acid for pH stabilization, and calcium stearate as a lubricant. Peppermint and eucalyptus oils were included in selected formulations to enhance organoleptic properties.

Table 1

Optimized formulations for MVSHS-40 tablets (per tablet, mg)

Component	F1	F2	F3	F4	F5
Dry extract MVSHS-40	50.0	50.0	50.0	50.0	50.0
MCC 105	75.0	75.0	75.0	75.0	75.0
EMDEX	–	593	589.5	589	587.5
Citric acid	28.0	28.0	28.0	28.0	28.0
Calcium stearate	–	4.0	7.5	7.5	7.5
Lactose monohydrate	589.5	–	–	–	–
Peppermint oil	–	–	–	–	1.5
Eucalyptus oil	–	–	–	0.5	0.5
Tablet weight (mg)	750.0	750.0	750.0	750.0	750.0

The use of EMDEX improved flow and compressibility. Inadequate tablet ejection observed with 4 mg calcium stearate prompted an increase to 7.5 mg. MCC enhanced water absorption and disintegration, while essential oils improved flavor.

The tablets were evaluated for mass uniformity, disintegration, dissolution, mechanical strength, and friability, complying with SPRK standards. Formulation 5 was deemed optimal.

Table 2

Quality evaluation of MVSHS-40 tablets

Test	Requirement	F1	F2	F3	F4	F5
Appearance	Conforms	✓	✓	✓	✓	✓
Avg. weight variation	±5 %	0.002	0.004	0.002	0.003	0.003
Mass uniformity	±10 %	3	4	4	2	5
Disintegration time	≤15 min	13:00	12:35	12:55	12:45	11:47
Dissolution (45 min)	≥75 %	72 %	81 %	80 %	79 %	79 %
Crushing strength (N)	≥50 N	151.8	139.5	142.1	135.4	107.2
Friability	≥97 %	99.994	99.995	99.993	99.994	99.991

Bulk and tapped density, flow rate, compressibility, and ejection force were evaluated (Table 3).

Table 3

Physico-technological properties of MVSHS-40 tablet blend

Property	Value
Bulk density (g/cm³)	0.53 ± 0.01
Tapped density (g/cm³)	0.75 ± 0.02
Flow rate (g/s)	3.1 ± 0.1
Compressibility (kg)	8.5 ± 0.21
Compression index (%)	3.97
Ejection force (kg/cm²)	270.0 ± 8.0

The blend exhibited good flow and compressibility, suitable for direct compression.

Tablets were produced using direct compression. Weighing of MVSHS-40 and excipients was performed using MK-32.2 and ET-600P-E scales. All components were sieved through a PVS30 vibrosieve and mixed in an Airpac blender. Lubrication was performed by adding calcium stearate, followed by mixing for 5 minutes. Moisture content was controlled (5-6 %) using EVLAS-2M. Tablets were compressed using a rotary tablet press RTM-10 and then dedusted using an Elevating De-Duster.

Direct compression provided several advantages over wet granulation, including process simplicity, reduced equipment use, and lower labor and energy costs. The technological flowchart for the production of tablets with MVSH-40 by the direct compression method is presented in Figure 1.

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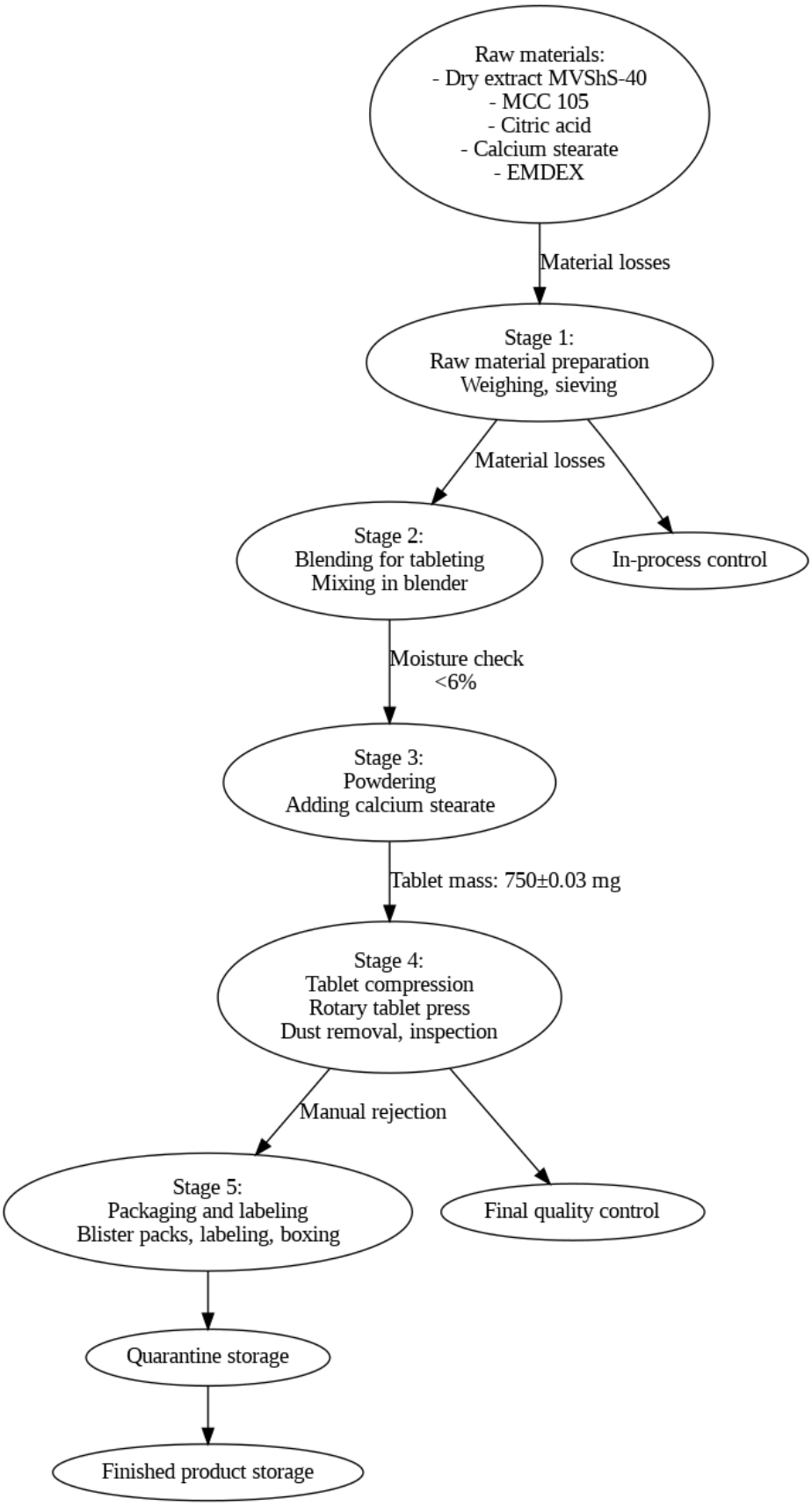
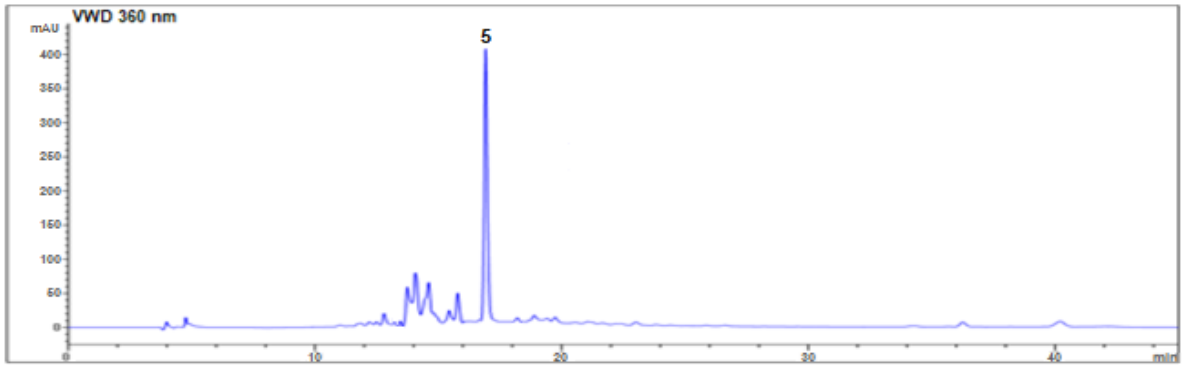


Figure 1. Technological flowchart for the production of tablets with MVSH-40.

HPLC-UV analysis confirmed the presence of rosmarinic acid, quantified as 0.41 % (3.046 mg per tablet). Chromatograms showed clear identification peaks under multiple detection wavelengths (Fig. 2).



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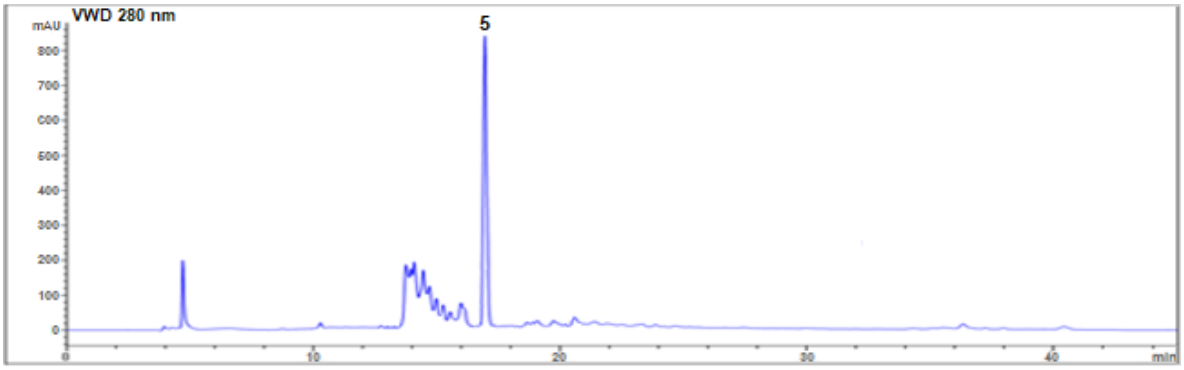

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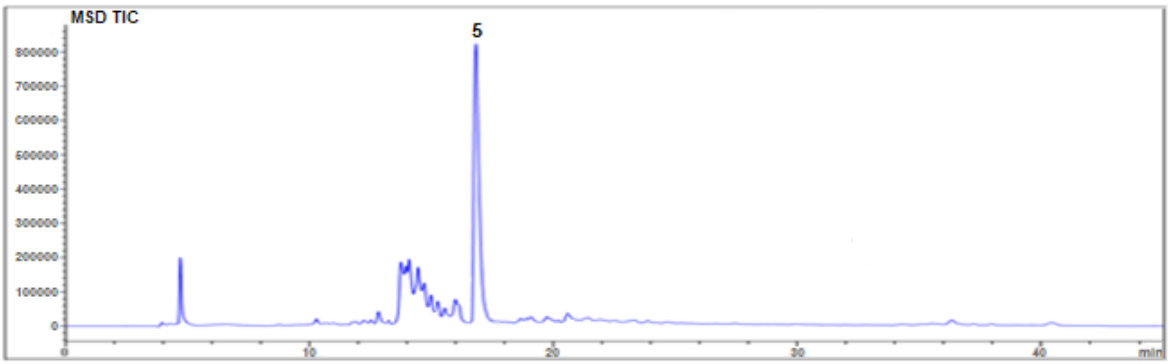
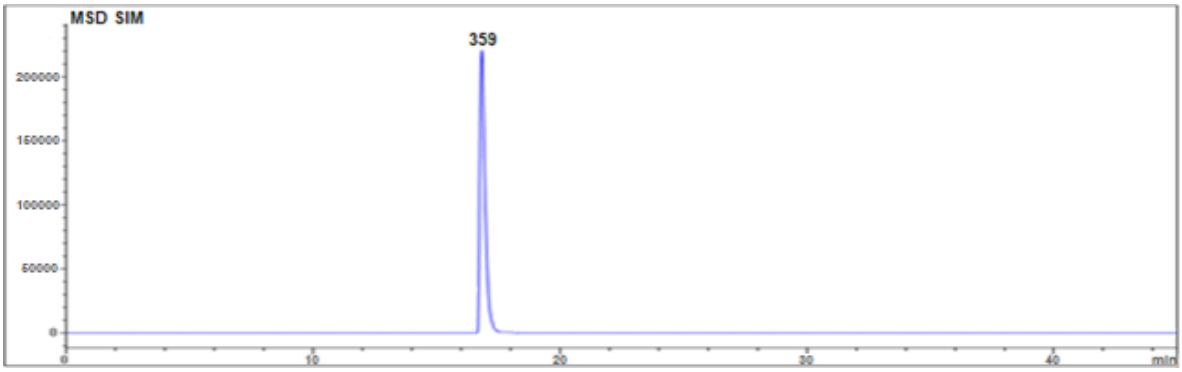


C



D

Figure 2. HPLC-UV chromatograms: A – 360 nm, B – 280 nm; HPLC-MS/MS: C – total ion chromatogram (TIC), and D – identification of rosmarinic acid in a tablets with steppe sage extract (SIM).

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A comparison with commercial *Salvia officinalis* tablets (Netherlands) highlighted key advantages of the MVSHS-40 tablets (Table 4).

Table 4

Comparative characteristics of Salvia-based tablets

Parameter	*Salvia officinalis*	*Salvia stepposa* (MVSHS-40)
Extract type	Dry	Dry
Active content	12.5 mg	50 mg
Essential oils	Sage essential oil	Eucalyptus, peppermint oils
Rosmarinic acid content	0.55 mg	3.046 mg
Safety concern	Contains thujone	Thujone-free

MVSHS-40 tablets offer significantly higher active content without the safety concerns associated with thujone.

Long-term stability studies were conducted over 18 months at 25±2 °C/60±5 % RH. Three production batches were evaluated at 0, 3, 6, 9, 12, and 18 months. No significant changes in physical or chemical parameters were observed, confirming product stability.

Quality specification was developed in compliance with EAEU and SPRK guidelines, encompassing organoleptic, physicochemical, microbiological, and assay parameters.

Table 5

Quality specification for tablets based on MVSH-40

Quality parameter	Specification (acceptable limits)	Test method references
Description	Tablets whole, uniform, light gray with light brown specks, homogeneous surface, bitter taste, characteristic aromatic odor.	EAEU Pharm. 2.1.6.0, SPRK Vol.1, p.550
Identification	Retention time of main peak in test solution chromatogram must match rosmarinic acid peak in standard chromatogram.	According to RD
Average mass / mass uniformity	±5 % individual tablet, ±10 % allowed deviations	EAEU Pharm. 2.1.9.5, SPRK Vol.1, 2.9.5
Disintegration	Not more than 15 minutes	EAEU Pharm. 2.1.9.1, SPRK Vol.1, 2.9.1
Dissolution	Not less than 75 % dissolved in 45 minutes	EAEU Pharm. 2.1.9.3, SPRK Vol.1, 2.9.3
Related substances (identified impurities)	Caffeic acid, cynaroside, p-coumaric acid, apigenin	According to RD
Friability	Not less than 97 %	EAEU Pharm. 2.1.9.6, SPRK Vol.1, 2.9.7
Tablet crushing strength	Not less than 50 N	EAEU Pharm. 2.1.9.7, SPRK Vol.1, 2.9.8
Microbiological purity	Category 3B. In 1.0 g: aerobic microorganisms ≤10*4, fungi ≤10*2, enterobacteria ≤10*2. No *Escherichia coli* in 1.0 g, *Salmonella* in 10.0 g, *Staphylococcus aureus* in 1.0 g allowed.	EAEU 2.3.1.4, SPRK Vol.1, 2.16.12, 2.16.13, 5.1.4
Loss on drying / Water content	Not more than 6.5 %	EAEU Pharm. 2.1.2.31, SPRK Vol.1, 2.2.32
Quantitative determination	Not less than 1.5 mg per tablet	According to RD
Packaging	Blister pack of polyvinyl chloride film and printed lacquered aluminum foil or heat-sealable packaging paper, 10 tablets per blister. 2, 3, or 5 blister packs per carton.	According to RD
Labeling	Label indicates name and content of active substances, expiry date, storage conditions, usage instructions.	According to RD
Transportation	According to GOST 17768-90E	GOST 17768-90E
Storage	In a place protected from light, at temperature not exceeding 25 °C	According to RD
Shelf life	18 months (observation period)	According to RD
Main pharmacological action	Anti-inflammatory, antimicrobial	According to RD

Conclusion

The development of tablets containing a dry extract MVSHS-40 of *Salvia stepposa* demonstrated promising results as a natural antimicrobial and anti-inflammatory agent suitable for pharmaceutical use. MAE using 40 % ethanol efficiently yielded a dry extract rich in bioactive compounds, including rosmarinic acid at 6 % of extract mass. The tablets, formulated by direct compression with optimized excipients, showed excellent physico-technological properties: uniform mass with variation within ±5 %, rapid disintegration times below 12 minutes, and dissolution rates above 79 % within 45 minutes, meeting pharmacopeial standards.

Quantitative HPLC-UV/MS analysis confirmed the presence of 3.046 mg of rosmarinic acid per 50 mg tablet of dry extract, significantly surpassing comparable commercial *Salvia officinalis* tablets (0.55 mg rosmarinic acid per tablet). Mechanical testing showed tablets had robust crushing strength (≥107 N) and low friability (≥99.99 %), ensuring integrity during handling and transport. Long-term stability studies over 18 months at controlled temperature and humidity demonstrated no significant changes in quality attributes, supporting product shelf life.

The absence of thujone, a toxic compound found in some *Salvia* species, further enhances the safety profile of MVSHS-40 tablets. Given their potent antimicrobial activity against key pathogens (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa,* and *Candida albicans*) and excellent pharmaceutical qualities, these tablets represent a viable natural alternative for upper respiratory tract infection treatments. This study confirms that MVSHS-40 tablets combine efficacy, safety, and stability, warranting further clinical evaluation and potential commercial production.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Levaya Ya.K**. – Investigation, Methodology, Development of formulation and technology, Writing-review & Editing; **Atazhanova G.A**. – Conceptualization, Data curation; **Badekova K.Zh**. – Methodology, Plant material collection; **Ivasenko S.A**. – Data curation, HPLC-UV/MS.

Conflict of Interest

Authors declare no conflict of interest.

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Salvia stepposa таблеткаларын фармацевтикалық әзірлеу және стандарттау

Антимикробтық төзімділіктің артуына байланысты, өсімдік текті қосылыстар дәстүрлі антибиотиктерге қауіпсіз және тиімді балама ретінде кеңінен зерттелуде. Бұл зерттеуде микробқа қарсы және қабынуға қарсы белсенділігі дәлелденген фенол қышқылдары мен флавоноидтарға бай *Salvia stepposa* құрғақ экстракт (MVSHS-40) негізінде қатты дәрілік форманы әзірлеу жүргізілді. Экстракт микротолқындық экстракция (MAE) әдісімен 40 % этанол қолданып алынған, бұл 6 %-ға дейін розмарин қышқылын бөліп шығаруға мүмкіндік берді. Таблеткалар микроцеллюлоза, EMDEX, лимон қышқылы және кальций стеараты қосылған және кейбір нұсқаларға эфир майлары енгізілген тікелей таблеткалау әдісі арқылы дайындалды. 30-дан астам эксперименталды нұсқаның ішінен 5 оңтайландырылған құрамы таңдалды. Физика-химиялық және фармакопеялық сынақтар таблеткалардың дисперсиялану уақыты 12 минуттан аз, ерігіштік көрсеткіші 79 %-дан жоғары, салмақтың біркелкілігі, жоғары механикалық беріктік (≥107 Н) және төмен сынғыштық (≥99.99 %) екенін растады. HPLC-UV/MS талдауы бойынша бір таблеткада 3.046 мг розмарин қышқылы бар екені анықталды. Саудаға арналған *Salvia officinalis* таблеткаларымен салыстырғанда, MVSHS-40 таблеткаларында белсенді заттың мөлшері 5,5 есе жоғары болып, туйонмен байланысты қауіпсіздік мәселелерінен сақталған. 18 ай бойы жүргізілген тұрақтылық зерттеулері препараттың фармакологиялық қасиеттерінің өзгермейтінін көрсетті. Алынған MVSHS-40 таблеткалары барлық сапа талаптарына сәйкес келеді және жоғарғы тыныс жолдарының инфекцияларының алдын алу және емдеу үшін қолдану мүмкіндігі бар қауіпсіз, тұрақты және тиімді өсімдік негізіндегі дәрілік форма.

Кілт сөздер: Salvia stepposa, микротолқынды экстракция, розмарин қышқылы, фенолды қосылыстар, HPLC-MS/MS, фитофармацевтика.

Я.К. Левая, К.Ж. Бадекова, Г.А. Атажанова, С.А. Ивасенко

Фармацевтическая разработка и стандартизация таблеток из *Salvia stepposa*

В связи с возрастающей угрозой антимикробной резистентности растительные соединения всё чаще рассматриваются как безопасная и эффективная альтернатива традиционным антибиотикам. В данном исследовании была проведена разработка твердой лекарственной формы на основе сухого экстракта *Salvia stepposa* (MVSHS-40), богатого фенольными кислотами и флавоноидами с доказанной антимикробной и противовоспалительной активностью. Экстракт получали методом микроволновой экстракции (MAE) с использованием 40 % этанола, что позволило выделить до 6 % розмариновой кислоты. Таблетки формировали методом прямого прессования с применением микрокристаллической целлюлозы, EMDEX, лимонной кислоты и кальция стеарата; в некоторых вариантах добавляли эфирные масла. Из более чем 30 экспериментальных вариантов было отобрано пять оптимизированных составов. Физико-химические и фармакопейные исследования подтвердили отличное время диспергирования (<12 минут), высокую скорость растворения (>79 %), однородность массы, высокую механическую прочность (≥107 Н) и минимальную ломкость (≥99,99 %). Анализ методом ВЭЖХ-УФ/МС показал содержание 3,046 мг розмариновой кислоты в одной таблетке. Сравнительный анализ выявил 5,5-кратное превышение содержания активного вещества по сравнению с коммерческими таблетками *Salvia officinalis* при отсутствии опасений, связанных с туйоном. Исследования стабильности в течение 18 месяцев продемонстрировали сохранение фармакологических свойств препарата. Полученные таблетки MVSHS-40 соответствуют всем требованиям качества и представляют собой безопасную, стабильную и эффективную фитопрепаративную форму с потенциалом применения для профилактики и лечения инфекций верхних дыхательных путей.

Ключевые слова: *Salvia stepposa,* микроволновая экстракция, розмариновая кислота, фенольные соединения, ВЭЖХ-МС/МС, фитофармацевтика.

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Development of micropropagation protocol for production of elite blueberry planting material in Kazakhstan

This study presents an optimized protocol for micropropagation of commercially valuable highbush blueberry (*Vaccinium corymbosum* L.) varieties adapted to climatic conditions of Kazakhstan. An effective explant surface disinfection technique was established, providing a high percentage of aseptic plants. Various nutrient media with auxins (IBA, IAA) and cytokinins (zeatin, BAP) were evaluated for shoot proliferation and rooting. The optimal medium for micropropagation was identified as Woodt Plant Medium (WPM) with a double concentration of $Ca(NO_3)_2$, 2 mg/L zeatin, 30 g/L sucrose, 3.5 g/L agar, 1.5 g/L gelzan, pH 5.2. The most effective shoot rooting was achieved on half-strength WPM medium with 0.5 mg/L IBA, 30 g/L sucrose, and vermiculite (pH 5.2). *In vitro* rooted plants were successfully acclimatized to soil conditions by gradually reducing humidity in a controlled greenhouse environment at 20–23 °C. The developed micropropagation protocol enables rapid and mass propagation of elite blueberry varieties, ensuring genetic stability and minimizing the risk of pathogen infection. The results obtained contribute to the development of industrial blueberry cultivation in Kazakhstan and promote of modern biotechnological approaches in berry crop production.

Keywords: blueberry, *Vaccinium corymbosum* L., micropropagation, *in vitro* rooting.

Introduction

Highbush blueberry (*Vaccinium corymbosum* L.) belongs to the *Ericaceae* family and is a promising berry crop showing a steady growth in global consumption and expansion of cultivation areas [1-2]. In recent years, global blueberry production has been increasing at an average annual rate of 10 %, reaching nearly 2 million tons in 2023, more than double the five-year average. The high economic and biological value of blueberries is due to the rich content of biologically active substances, including polyphenols, combined with low caloric content and excellent taste [1]. Blueberry is used to strengthen blood capillaries, its positive effect on the thyroid gland has been revealed; berries have antisclerotic, anti-inflammatory and antitumor effects [3].

Based on information from Kazakhstan's Ministry of Agriculture, the current production of fruits and berries in the country is about 420 thousand tons, but more than 2.5 million tons are required to fully meet the needs of the population. In this regard, about 70 % of fruit and berry products are imported, which emphasizes the need to develop domestic production of berry crops, including blueberries.

Traditional methods of vegetative propagation (green and woody cuttings) are widely used in nursery farming, but their efficiency is limited by low rooting rate and accumulation of intracellular pathogens, which negatively affects the quality of berry products and leads to significant yield losses. In this regard, micropropagation is the most effective method that allows to quickly and massively obtain healthy and high-quality plants, reduce the risks of pathogen spread, maintain genetic homogeneity and obtain high-quality planting material.

Scientific research in the field of micropropagation of blueberry is actively carried out in the USA [4, 5], Russia [6], Belarus [3, 7], EU countries [8, 9]. In Kazakhstan, studies on micropropagation of blueberry were initiated at the Kazakh National Agrarian University, but they included only the development of the mode of sterilization of explants and selection of nutrient medium at the step of *in vitro* culture initiation, while the issues of propagation and *in vitro* shoots rooting, as well as obtaining planting material with a closed root system were not considered in this article [10].

The purpose of this work is to develop biotechnology of accelerated micropropagation of blueberry and obtaining elite planting material of promising for Kazakhstan berry culture for further implementation in the practice of nursery farming.

Materials and Research Methods

Plant Material and Conditions of In Vitro Culture. Commercially valuable blueberry varieties were used as research objects: Bluecrop, Blue Gold, Chandler, Duke, Legacy, Meader, Spartan (USA).

The *in vitro* culture method was used to initiation *in vitro* plant material and micropropagation of berry plants [11].

To initiate *in vitro* culture of blueberry varieties, shoots (15–20 cm long) from container-grown plants were washed with soapy water, rinsed, cut into 1.0–1.5 cm pieces, disinfected in 0.1 % mercuric chloride for 3–10 minutes, and rinsed three times with sterile distilled water. Microcuttings were placed in tubes with various variants of Woody Plant Medium (WPM) supplemented with growth regulators: zeatin, 6-benzylaminopurine (BAP), indole-3-butyric acid (IBA) and gibberellic acid (GA) (Table 1).Composition of WPM medium (mg/L): $NH_4NO_3$400, H_3BO_3 6.2, $CaCl_2$ 72.5, $Ca(NO_3)_2$386, $CuSO_4$•$5H_2O$ 0.25, Na_2 EDTA•$2H_2O$ 37.3, $FeSO_4$•$7H_2O$ 27.85, $MgSO_4$180.7, $MnSO_4$•H_2O 22.3, Na_2MoO_4•$2H_2O$ 0,25, $KH_2PO_4$170, $K_2SO_4$990, $ZnSO_4$•$7H_2O$ 8.6, Gelzan^{TM}1.5, Agar 3.5, pH 5.2 [12].

The number of green shoots, as well as shoots with bacterial and/or fungal contamination and necrosis were counted. After 4 weeks, *in vitro* green plants, without visible signs of contamination, were tested on selective medium 523 for the presence of endophytic bacteria [13]. Composition of the 523 medium: sucrose 10.0, casein hydrolysate 8.0, yeast extract 4.0, KH_2PO_4 2.0, $MgSO_4$•$7H_2O$ 0.15, Gelzan^{TM} 6.0, pH 6.9. Only aseptic plants were used for subsequent micropropagation. *In vitro* plants were cultured at 24 °C, 25 μmol•м$^{-2}$•с$^{-1}$light intensity, 16-h photoperiod, and transferred to fresh medium every 4 weeks.

Table 1

Variants of media used for blueberry micropropagation

Nutrientmedia	Growth regulators concentration(mg/L)	pH	Reference
Modified WPM with doubled concentration of Ca $(NO_3)_2$	Zeatin2.0	5.2	[14]
Standard WPM	Zeatin 2.0.+ IBA0.2	5.8	[15]
Standard WPM	BAP 0.1	5.2	-
Standard WPM	BAP 0.1 + GA 0.4	5.2	-
Standard WPM	BAP 0.05	5.2	-
Standard WPM	BAP 0.05 + GA 0.4	5.2	-

The multiplication rate was calculated by the formula (1):

$$Mr = a/b \bullet c, \quad (1)$$

a — the number of newly formed shoots

b — the number of shoots transferred for micropropagation

c — number of subcultures

Rooting of *in vitro* plants was carried out using WPM medium with vermiculite, with half the mineral salts, and with 0.5 mg/L IBA, pH 5.2. 10 g of agrotechnical vermiculite (BioMaster) were placed in culture vessels (Magenta boxes) and 85 ml of liquid WPM medium were poured in. The Magenta boxes prepared in this way were autoclaved at 0.8-1.0 atm for 20 min.

To obtain planting material, the *in vitro* plants with roots were transferred into soil substrate consisting of peat, chernozem and perlite in various combinations and transferred to the greenhouse. The effect of temperature and humidity in the greenhouse on plant establishment and adaptation was recorded.

The experiments were repeated in three replicates (n = 25–30). Data presented are means and standard deviations. Statistical analysis was performed according to generally accepted methods [16].

Results and Discussion

In vitro culture initiation and propagation of aseptic plants

The initiation of in vitro culture and obtaining aseptic plants represents the initial and essential step in developing micropropagation techniques. One of the important issues of this stage is the selection of effective disinfection of plant material introduced into in vitro culture, since the donor plants were grown in containers, i.e. in non-sterile conditions (Fig. 1).

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Figure 1. Donor blueberry plants used for *in vitro* culture initiation

For disinfecting plant material, a 0.1 % solution of mercuric chloride was used. During the initiation of *in vitro* culture of blueberry varieties, it was observed that shoot apices were sensitive to this disinfectant. A 10-minute exposure resulted in complete necrosis of all explants. Reducing the treatment time to 7 minutes led to necrosis in 40–50 % of the apices. However, shortening the duration to 4 minutes significantly improved outcomes, promoting the development of green shoots. Under this treatment, bacterial and fungal contamination occurred in only 3.2–10.3 % of explants, necrosis ranged from 0 to 34.2 %, and green shoot formation was achieved in 39.4–93.7 % of explants, depending on the variety. The Duke variety showed the highest necrosis rate (34.2 %), while the Meader variety produced the highest number of green plants (93.7 %) (Fig. 2).

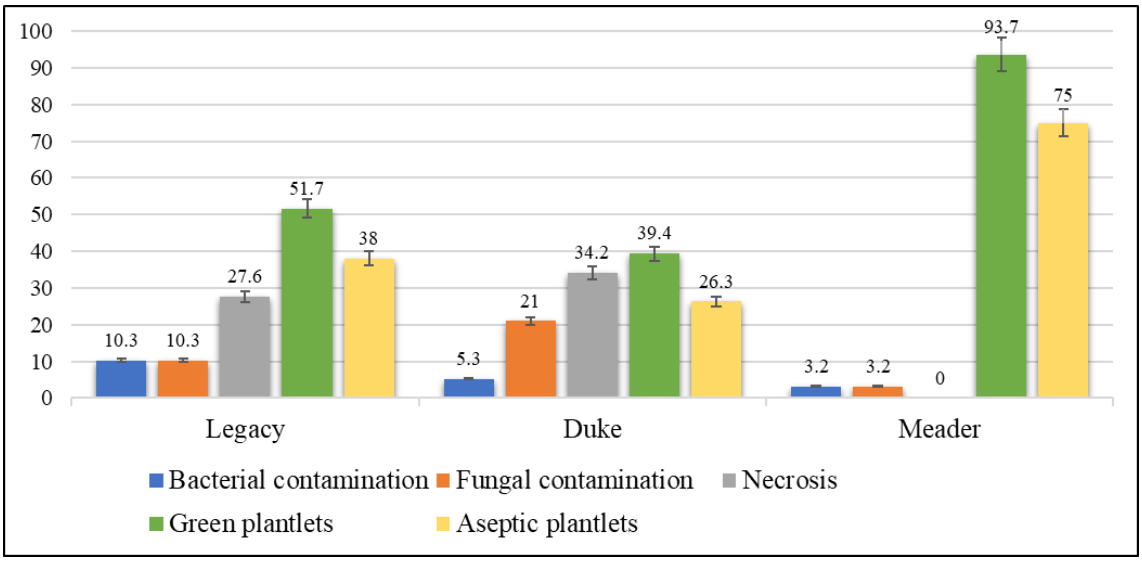
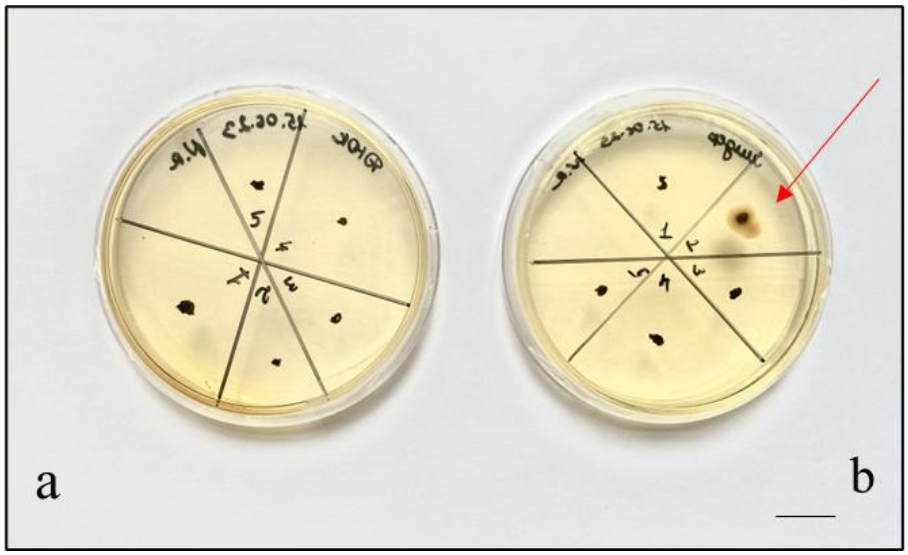


Figure 2. *In vitro* culture initiation results for the blueberry cultivars Legacy, Duke, and Meader

In vitro plant testing for endophytic contamination

An important stage of this study was to test plants for endophytic bacterial contamination. Four weeks after *in vitro* culture initiation, green plants without visible signs of contamination were tested on selective medium 523 to detect endophytic bacteria [13]. During passaging, basal parts of shoots were placed in Petri dishes with 523 medium. The development of bacterial contamination on selective medium 523 indicated endophytic contamination of in vitroplants (Fig. 3).



Arrows indicate bacterial contamination on the basal parts of in vitro shoots
Scale 1 cm.

Figure 3. Detection of endophytic bacterial contamination in tissues of Duke (a) and Meader (b) blueberry varieties on 523 selective medium

In vitro plants showing endophytic bacterial contamination were discarded. Following contamination screening, aseptic plants of seven blueberry varieties were successfully obtained. The proportion of aseptic plants ranged from 26.3 % to 75 %, depending on the variety, and these plants were suitable for further micropropagation (Fig. 4).

Scale 1 cm.

Figure 4. Aseptic shoots of Meader blueberry variety suitable for further micropropagation

It is noteworthy that this method of testing plant material for endophytic contamination is not consistently applied in other studies [10, 14, 17–19]. However, this step is crucial for ensuring the production of healthy planting material and is also essential for successful cryopreservation and long-term storage of plant genetic resources in cryobanks.

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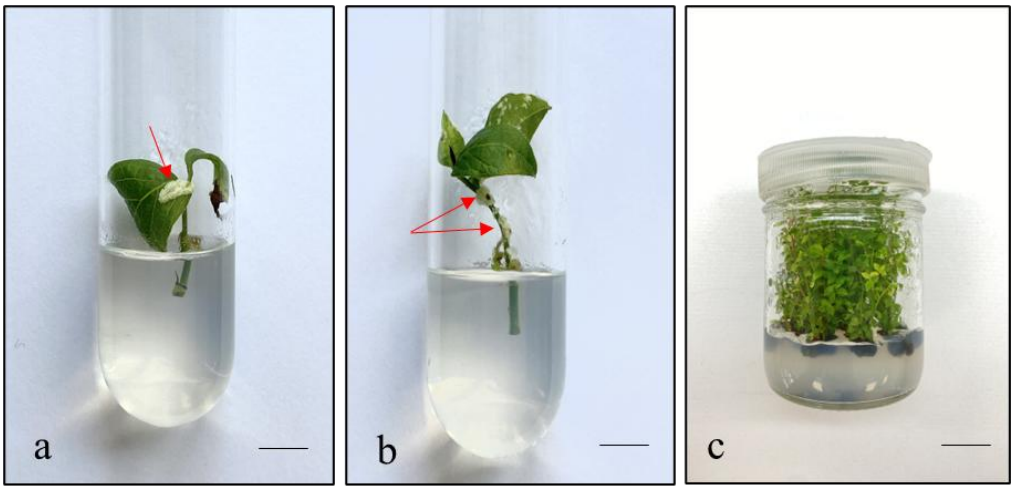
Micropropagation of blueberries

An equally important stage in the micropropagation process is optimizing the composition of the nutrient medium. For blueberries, the goal of nutrient medium selection was to maximize the multiplication rate (Mr) and ensure optimal plant quality—characterized by bright green leaves, absence of pigmentation or necrosis on leaves and shoots, and no callus formation. Phytohormones as BAP, IBA, GA are the main growth regulators used in *in vitro* blueberry propagation [14, 17]. In studies conducted by Reed B.M., it was demonstrated that the use of modified WPM medium with double concentration of $Ca(NO_3)_2$ and supplemented with zeatin significantly enhanced plant quality and Mr compared to media containing BAP as cytokinin [14].

Six variants of nutrient media based on the WPM with various plant regulators—zeatin, BAP, IBA, and GA were tested for blueberry micropropagation (Table 1). The results showed that excessive callus formation on leaves and shoots was the main limiting factor in the micropropagation process. This issue was especially evident when using WPM medium supplemented with BAP, even at a low concentration of 0.1 mg/L (Fig. 5). Replacing BAP with zeatin at 2.0 mg/L significantly improved shoot quality, eliminated callus formation, and increased the multiplication rate (Mr) (Fig. 5).



Arrows indicate callus formation on leaves and shoots.
Scale1 cm.

Figure 5. Blueberry shoots of Blue Gold variety on WPM medium with 0.1 mg/L BAP (a, b), shoots of Meader variety on WPM medium with 2.0 mg/L zeatin (c)

The Mr of blueberry varieties on the modified WPM medium varied from 6.3 for the Spartan variety to 8.5 for the Meader variety, and on average for all varieties was 7.5 (Table 2).

Table 2

Multiplication rate, rooting percentage and adaptation percentage of blueberry *in vitro* plants

Cultivar	Mr*	Rooting**, %	Adaptation***, %
Bluecrop	6.6±0,89	96.3	92.0
Blue Gold	8.1±0.76	92.6	100
Chandler	7.2±0.53	100	96.6
Duke	8.1±0.31	96.7	96.6
Legacy	7.5±0.23	92.6	92.0
Meader	8.5±0.31	100	100
Spartan	6.3±0.31	88.9	88.0
Mean	7.5±0.82	95.3±3.37	95.0±3.74

*Note**. n = 30 (3 x 10)
*Note***. n = 27–30 (3 x 9-10)
*Note****. All rooted plants were transferred for adaptation, n = 25–30

In the article by V. Litwińczuk [17], micropropagation of blueberry was carried out in two stages, differing in the content of phytohormones. At the first stage, cytokinin zeatin (0.5-1.0 mg/L) was used during *in vitro* culture in 2-3 passages, which was replaced by cytokinin 2-isopentyladenine (2-iP) (5–10 mg/L) in subsequent propagation. This approach was used by the author to reduce the cost of purchasing the expensive hormone zeatin, although some authors [16] have noted that zeatin is a more suitable phytohormone for *in vitro* culture initiation and micropropagation of blueberry. In this study, a high Mr of blueberry cultivars was also achieved on medium with zeatin (Fig. 5, c).

In vitro rooted plants and adaptation to soil substrate

The aseptic blueberry plants obtained were propagated in sufficient numbers to carry out the subsequent shoot rooting step. The in vitro rooting process is a difficult step, especially for hard-to-root species. Several variants of nutrient medium with auxins: IBA and indole-3-acetic acid (IAA) were tested for rooting blueberries.

Earlier foreign studies have shown that *ex vitro* rooting can be carried out both without pretreatment with auxins and after a short-term immersion in the IBA solution [18]. This method helps lower the cost of plant propagation; however, its effectiveness is limited by a reduced root formation rate compared to in vitro rooting. Previous studies have shown that in vitro rooting reduces the risk of disease and enhances resistance to environmental stress factors [19].

In the present work, an improved method of rooting blueberry shoots was applied based on the use of vermiculite added to the medium instead of agar, which ensured a high percentage of rooting of blueberry *in vitro* shoots (Table 2), as well as higher adaptability of plants when transferred to a soil substrate (Fig. 6, a). The optimal medium for shoot rooting was determined to be half-strength WPM containing 0.5 mg/L IBA, 30 g/L sucrose and vermiculite at pH 5.2. The rooting percentage varied from 88.9 % for the Spartan variety to 100 % (Meader, Chandler), and the average for varieties was 95.3 % (Table 2).

The rooted plants in *in vitro* culture were transplanted into soil substrate consisting of mixture of black and brown peat: perlite (9:1). Within a week, plants were adapted to reduced humidity by removing the lids from the cultivation containers. Temperature and humidity were monitored daily in the greenhouse where the plants were adapted. The optimal temperature for transferring aseptic plants to soil is 20–23°C.

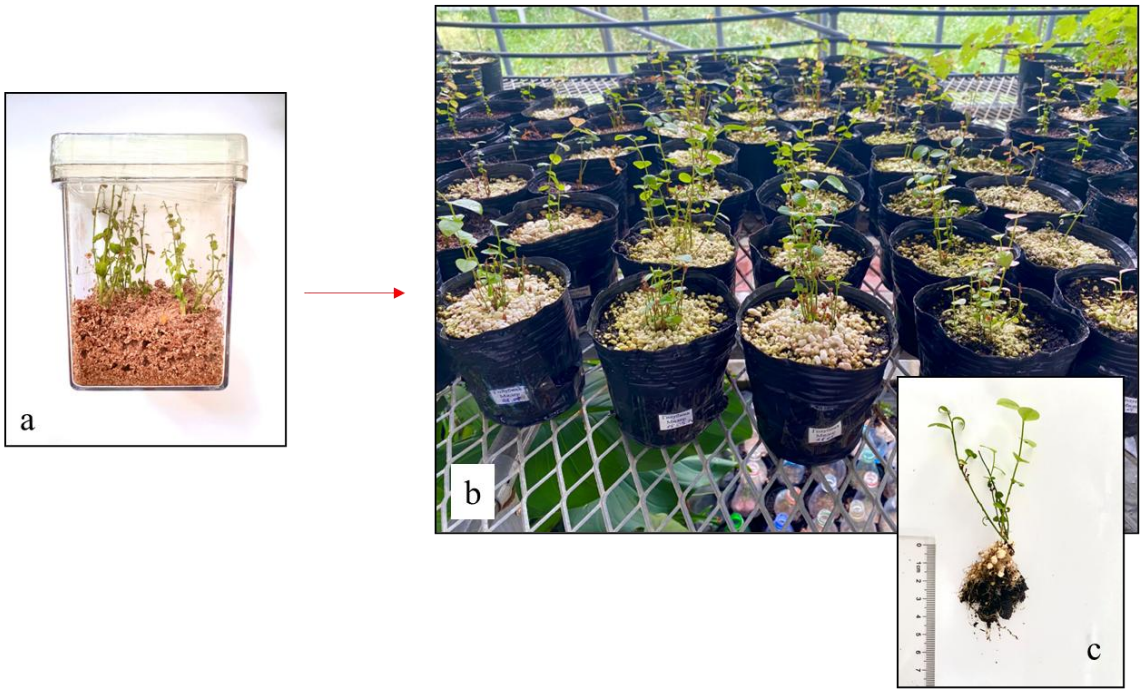


Figure 6. a) Shoot rooting on half-strength WPM medium containing 0.5 mg/L IBA, 30 g/L sucrose and vermiculite, pH 5.2. b) Blueberry plants in container culture in the greenhouse c) rooted blueberry plant (Meader variety)

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Due to the fact that blueberry grows best in peat soils, the following composition of soil substrate was used: a mixture of black and brown peat: perlite (9:1), a small depression was made in it for the volume of the root system and filled with perlite, where the plant was transplanted. As a result, on average 95.0 % of blueberry plants adapted to the soil substrate (Table 2).

Based on the conducted research, a protocol for accelerated *in vitro* propagation of plants of commercially valuable blueberry varieties was developed (Fig. 7)

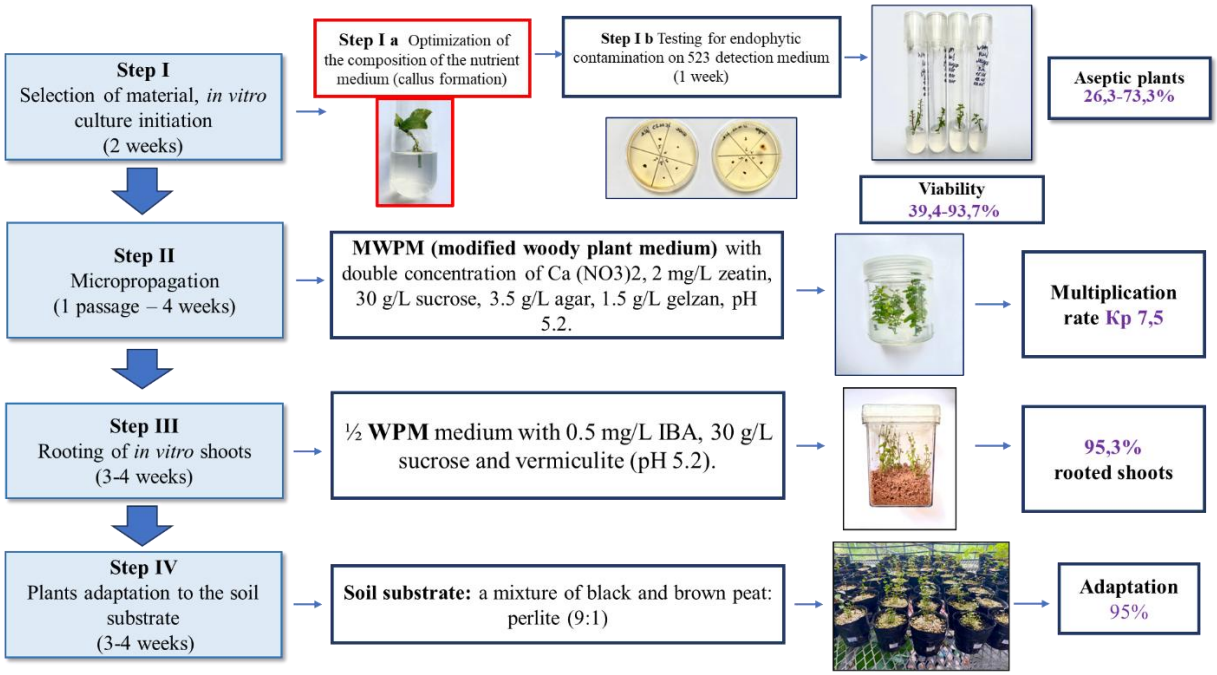

Figure 7. Protocol for micropropagation of blueberry varieties

The developed protocol for accelerated *in vitro* propagation of commercially valuable blueberry varieties includes a number of consecutive stages: selection of plant material, *in vitro* culture initiation of shoot apices, disinfection from epiphytic and endophytic fungal and bacterial contamination, micropropagation and rooting of *in vitro* shoots, *in vitro* plants adaptation to soil substrate

Conclusion

As a result of this research, an effective method of micropropagation of highbush blueberry (*Vaccinium corymbosum* L.) has been developed. Disinfection treatment for explants were optimized, providing a high percentage of aseptic plants. The most effective nutrient medium for micropropagation and rooting was established, which allowed to obtain healthy planting material with a high degree of rooting.

The developed technologies allow rapid and mass multiplication of promising blueberry varieties, reducing the risk of pathogen spread and increasing the genetic homogeneity of plants. The results obtained can be used for industrial cultivation of blueberries and introduction of modern biotechnological approaches in berry growing in Kazakhstan.

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Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Rymkhanova N.K.** – Conceptualization, Methodology, Investigation, Writing – original draft; **Manapkanova U.A.** – Investigation (micropropagation); **Mikhailenko N.V.** –

Investigation (rooting and plant adaptation); **Kushnarenko S.V.** – Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

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Қазақстанда жоғары сапалы көкжидек көшеттерін өндіру үшін микроклоналды көбейту әдісін әзірлеу

Зерттеуде Қазақстанның климаттық жағдайларына бейімделген, шаруашылықта құнды биік бұталы көкжидек (*Vaccinium corymbosum* L.) сорттарының микроклоналды көбейтуге арналған оңтайландырылған протоколы ұсынылды. Жұмыс барысында экспланттарды тиімді зарарсыздандыру әдістері әзірленіп, жоғары пайыздық асептикалық өсімдіктер қамтамасыз етілді. Өскіндерді микроклоналды көбейту және олардың тамырлануын қамтамасыз ету үшін ауксиндер (ИМК, ИУК)

мен цитокининдер (зеатин, БАП) қосылған түрлі қоректік орталар сыналды. Микроклоналды көбейту үшін оңтайлы орта ретінде қосарланған мөлшердегі $Ca(NO_3)_2$, 2 мг/л зеатин, 30 г/л сахароза, 3,5 г/л агар, 1,5 г/л джелрайт және pH 5,2 болатын WPM ортасы анықталды. Өскіндерді тамырландыруда ең жақсы нәтижелер ½ WPM ортасында, 0,5 мг/л ИМК, 30 г/л сахароза және вермикулит (pH 5,2) қолданылған жағдайда алынды. *In vitro* тамыры бар өсімдіктер 20–23°C температурада бақыланатын жылыжай ортасында ылғалдылықты біртіндеп төмендету арқылы топырақ жағдайына сәтті бейімделді. Әзірленген протокол көкжидектің элиталық сұрыптарын жаппай әрі жедел көбейтуге мүмкіндік береді, генетикалық тұрақтылықты қамтамасыз етеді және патогендермен зақымдану қаупін азайтады. Бұл нәтижелер Қазақстанда көкжидекті өнеркәсіптік өсіруді дамытуға және жидек шаруашылығында заманауи биотехнологиялық әдістерді енгізуге өз үлесін қосады.

Кілт сөздер: көкжидек, *Vaccinium corymbosum* L., микроклоналды көбейту, *in vitro* тамырландыру.

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Разработка способа микроклонального размножения для производства элитного посадочного материала голубики в Казахстане

В данном исследовании представлен оптимизированный протокол микроклонального размножения коммерчески ценных сортов голубики высокорослой (*Vaccinium corymbosum* L.), адаптированных к климатическим условиям Казахстана. Разработан эффективный способ дезинфекции эксплантов, обеспечивающий высокий процент асептических растений. Были протестированы различные питательные среды с добавлением ауксинов (ИМК, ИУК) и цитокининов (зеатин, БАП) для микроклонального размножения побегов и их укоренения. Оптимальная среда для микроклонального размножения оказалась среда WPM с удвоенной концентрацией $Ca(NO_3)_2$, 2 мг/л зеатина, 30 г/л сахарозы, 3,5 г/л агара, 1,5 г/л джелрайта, pH 5,2. Наилучшие результаты по укоренению побегов были получены при использовании WPM среды с половинной концентрацией минеральных компонентов, с 0,5 мг/л ИМК, 30 г/л сахарозы и вермикулитом (pH 5,2). Укоренённые *in vitro* растения были успешно акклиматизированы к почвенным условиям путем постепенного снижения влажности в контролируемой тепличной среде при температуре 20–23 °C. Разработанный протокол позволяет быстро и массово размножать элитные сорта голубики, обеспечивая генетическую стабильность и минимизируя риск заражения патогенами. Полученные результаты способствуют развитию промышленного выращивания голубики в Казахстане и внедрению современных биотехнологических методов в ягодоводство.

Ключевые слова: голубика, *Vaccinium corymbosum* L., микроклональное размножение, укоренение *invitro*.

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Comparative karyological analysis of four species of *Aedes* mosquitoes inhabiting the territory of the Republic of Kazakhstan

In the present study, karyotypic analysis of four species of *Aedes* mosquitoes (*Aedes vexans, Ae. caspius, Ae. cataphylla, Ae. subdiversus*) collected in the territory of the Republic of Kazakhstan was carried out. Chromosome length measurements were performed in each of the studied species, and C-staining, DAPI-fluorescence and fluorescence in situ hybridisation (FISH) methods were applied using 18S rDNA probe. C-staining and DAPI-fluorescence showed that regions of heterochromatin localized predominantly in the centromeric regions of chromosomes. FISH-hybridization results showed that in *Ae. cataphylla* the 18S rDNA loci are located on the first chromosome, whereas in *Aedes vexans, Ae. caspius* and *Ae. subdiversus* they were detected on the second chromosome.

Keywords: Aedes, bloodsucking mosquitoes, mitotic chromosomes, C-stain, DAPI, FISH, rDNA, heterochromatin.

Introduction

Mosquitoes of the *Culicidae* family represent the most numerous group of insects on the planet. These insects are widely distributed and can be found in both southern and northern regions. Mosquitoes are known carriers of a variety of infectious diseases caused by viral and parasitic pathogens, which significantly impact the health of both humans and animals. Many countries report imported cases of such diseases, while ongoing climate change contributes to the spread of disease vectors into previously unaffected areas. Among these expanding species, *Aedes* mosquitoes are commonly encountered [1–5].

The genus *Aedes* is characterized by high species diversity and includes numerous blood-feeding mosquito species [1]. One of the most invasive species of this genus, *Aedes koreicus*, was first identified in Kazakhstan in 2021 [6]. To effectively assess and mitigate potential epidemiological risks, it is essential to determine the precise species composition of local mosquito populations. However, species-level identification within the genus *Aedes* is often complicated due to morphological similarities among closely related taxa. As a result, the application of multiple diagnostic approaches is required to ensure accurate identification. Cytogenetic characteristics, such as the quantity and spatial organization of heterochromatin in chromosomes, are known to be species-specific in many animals and plants [7]. Among ribosomal genes, 18S rDNA is considered the most conserved, making it a valuable tool in taxonomic and phylogenetic research. In light of these considerations, a cytogenetic study was performed on selected *Aedes* species collected across Kazakhstan. The findings contribute to the development of additional taxonomic markers and offer insights into the evolutionary biology of *Aedes* mosquitoes.

Experimental

Fourth-instar larvae of *Aedes* mosquitoes were utilized in the present study. Field collections were carried out during spring 2024 in multiple regions of the Republic of Kazakhstan, including Karaganda, Kostanay, North Kazakhstan, and Akmola regions. The specimens represented various taxonomic groups: *Ae. vexans* (subgenus *Aedimorphus*), *Ae. caspius* (subgenus *Ochlerotatus*, caspius group), *Ae. cataphylla* (subgenus *Ochlerotatus*, communis group), and *Ae. subdiversus* (subgenus *Ochlerotatus*, rusticus group).

Sampling took place from March to May, with larvae collected from natural aquatic habitats and preserved in Carnoy's fixative (1 part glacial acetic acid to 3 parts 96 % ethanol). A taxonomic expert on blood-feeding mosquitoes performed morphological determinations using standardized identification protocols [8–10]. Analysis of larval characteristics was carried out with a Stemi 2000-C stereomicroscope (Carl Zeiss).

Cells obtained from the imaginal discs of *Aedes* larvae at the fourth instar stage were used to conduct cytogenetic analysis of four mosquito species: *Ae. vexans*, *Ae. caspius*, *Ae. cataphylla*, and *Ae. subdiversus*. This anatomical structure was selected due to the high density of cells undergoing metaphase, making it suitable for chromosome visualization. As in the majority of mosquito taxa, these species displayed a diploid chromosome number of 2n = 6. The Chromosomes classified according their length.

For chromosomal analysis, the lactoacetoorcein technique was employed to stain preparations, allowing for measurement of chromosome length, estimation of centromeric index, and calculation of relative lengths. To examine constitutive heterochromatin, C-banding was applied, while DAPI staining was used to detect A-T rich heterochromatin regions. Fluorescence in situ hybridization (FISH) enabled localization and quantification of 18S rDNA loci across *Aedes* chromosomes from different subgenera [11–14].

Chromosomal imaging and analysis were conducted using Zeiss Axio Imager A1 and Z1 fluorescence microscopes (Zeiss, Germany). Comparative karyotype studies employing Giemsa C-banding and related techniques have proven valuable for revealing patterns of chromosomal differentiation. These studies highlight the variation in heterochromatin quantity and distribution as key elements in mosquito genome evolution, which show contrasting features among others mosquitoes lineages [15, 16].

Results and Discussion

Studies focused on *Aedes* reveal variability in overall chromosome lengths [17]. Chromosomes were stained using the lactoacetoorcein technique (Fig. 1), allowing for precise measurement of their size using the ImageJ software (Table 1). Based on this, the relative chromosome length (Lr, %) and centromeric index (Jc, %) were calculated (Table 2). Notably, chromosome 1 in all species is significantly shorter than chromosomes 2 and 3, which are nearly identical in length within each species.

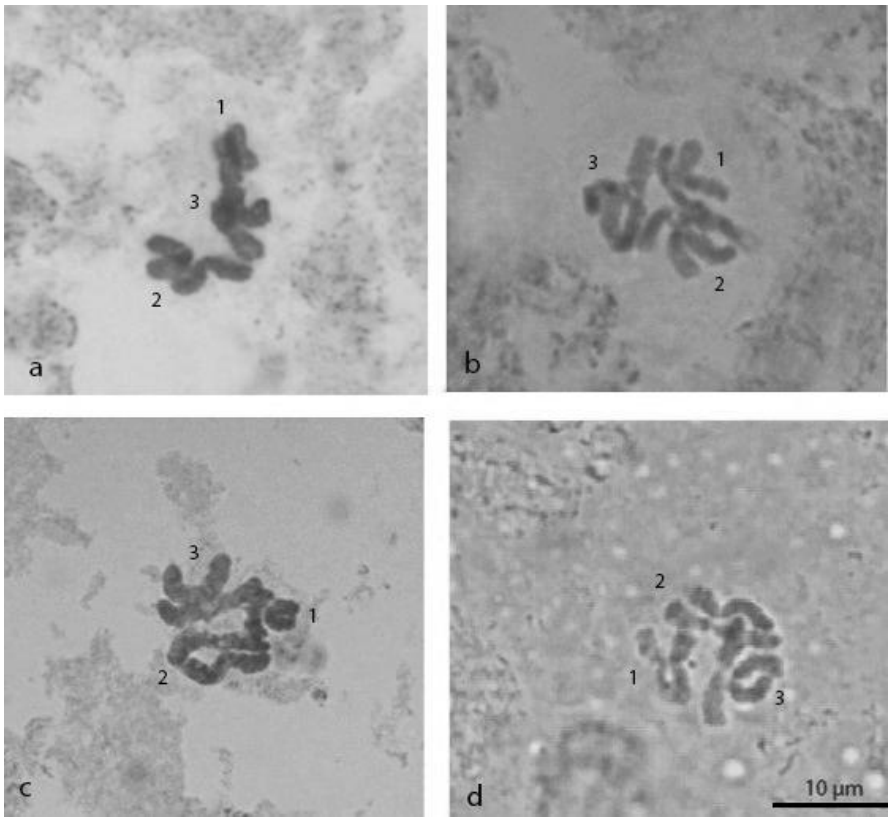

Figure 1. Lactoacetoorcein staining of chromosomes of *Ae.vexans* (a), *Ae.caspius* (b), *Ae.cataphylla* (c), *Ae.subdiversus* (d). 1,2,3 — chromosome numbers.

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Table 1

Chromosome lengths of mosquitoes of the genus Aedes

Species	Chromosome 1, ±0,3 μm	Chromosome 2, ±0,3 μm	Chromosome 3, ±0,3 μm
Ae.vexans	5.4	9.01	8.22
Ae.caspius	6.26	9.99	9.03
Ae.cataphylla	6.5	10.34	9.7
Ae.subdiversus	6.46	10.35	9.78

Centromeric index values were determined using the formula proposed in [18]. The observed relative lengths ranged from 24 % to 39 %, while centromeric indices fell between 45 % and 51 %, indicating that the chromosomes are metacentric, although chromosome 2 in some specimens approached submetacentric morphology.

Table 2

Centomeric index and relative length of chromosomes

Species	Chromosomes 1		Chromosomes 2		Chromosomes 3	
	L_r, %	J_c, %	L_r, %	J_c, %	L_r, %	J_c, %
Ae.vexans	24	48	39	50	36	49
Ae.caspius	25	48	39	46	35	48
Ae.cataphylla	24	48	38	47	36	47
Ae.subdiversus	24	48	39	45	36	47

C-banding analysis revealed the presence of constitutive heterochromatin in the centromeric areas of all examined species. As an illustration, Figure 2 shows heterochromatin patterns in *Ae. cataphylla*. Using DAPI staining, small A-T rich heterochromatin blocks were visualized, particularly in *Ae. vexans* and *Ae. subdiversus*. In contrast, these blocks were nearly undetectable in *Ae. caspius* and *Ae. cataphylla*. Figure 3 demonstrates the centromeric localization of DAPI-positive regions. These observations are consistent with previous works by Wasserlauf et al. (2018) and Alekseeva et al. (2020) [13, 14].

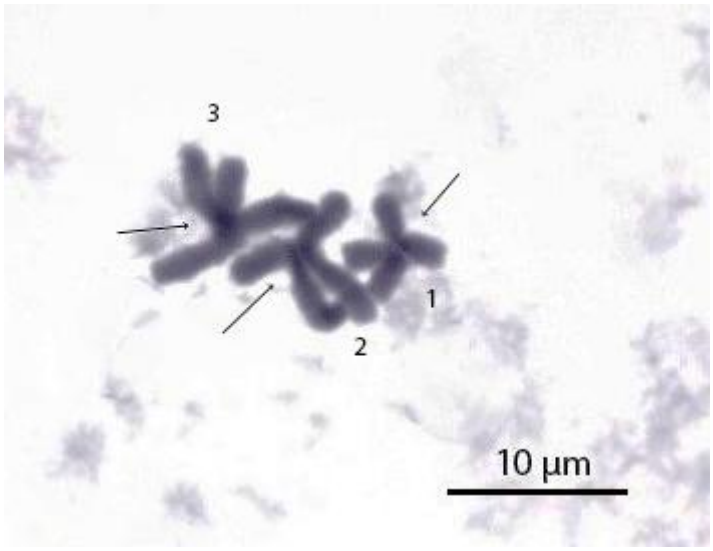

Figure 2. C-stained chromosomes in *Ae.cataphylla.*
Arrows indicate C-blocks of constitutive heterochromatin. The numbers indicate chromosome numbers.

Fluorescence in situ hybridization (FISH) was applied to all four studied species to map the 18S rDNA loci. All studied species have 1 loci 18S rDNA (Fig. 3).

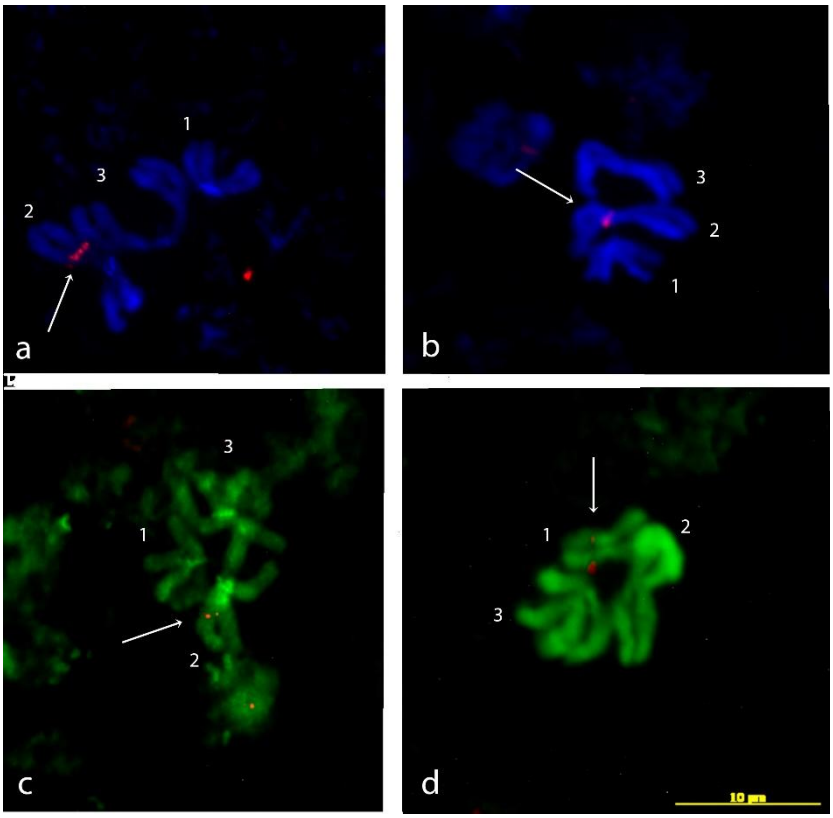

Figure 3. Localization of 18S rDNA on chromosomes of *Ae.vexans* (a), *Ae.caspius* (b) *Ae.subdiversus* (c) *Ae.cataphylla* (d). The arrows indicate the localization sites of 18S rDNA. The numbers indicate chromosome numbers. The chromosomes stained by DAPI. The colors of the chromosomes are artificial.

In *Ae. cataphylla*, the 18S rDNA cluster was located on chromosome 1, confirming earlier findings from specimens collected in the Tomsk region [19]. While *Ae. cataphylla* is part of the communis group within the *Ochlerotatus* subgenus — where 18S rDNA signals are typically observed on chromosome 2 [19] — our results show locus variability within this clade. *Ae. vexans* and *Ae. subdiversus*, which belong to different subgenera, exhibited 18S rDNA sites on chromosome 2 (Fig. 3).

Conclusions

The findings concerning chromosome size variation, as well as the distribution of heterochromatin and 18S rRNA gene loci, provide the additional cytogenetic markers for species differentiation within the *Aedes* genus and offer insights into their evolutionary history. The localization patterns of 18S rDNA in the species analyzed in this study (*Ae. vexans*, *Ae. caspius*, *Ae. subdiversus*, and *Ae. cataphylla*), and compared with earlier studies [19], reflect a common evolutionary pattern within groups of the *Ochlerotatus* subgenus. Observed differences in the chromosomal positioning of 18S rRNA genes — both between and within subgenera — suggest that evolutionary chromosomal rearrangements, such as translocations, have contributed to their relocation.

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Қазақстан Республикасы аумағында таралған *Aedes* туысына жататын масалардың төрт түрінің салыстырмалы кариологиялық талдауы

Зерттеуде Қазақстан Республикасының аумағында жиналған масалардың *Aedes* туысының 4 түріне (*Aedes vexans, Ae.caspius, Ae.cataphylla, Ae.subdiversus*) кариологиялық талдау жүргізілді. Нәтижесінде әрбір түрдің хромосомаларының ұзындығы өлшенді, С- және DAPI бояуы және 18S rDNA зондымен хромосомалардың флуоресцентті гибридизациясы (FISH) жүргізілді. С- және DAPI бояулары

центромера аймағында гетерохроматиннің локализациясын көрсетті. FISH Ae.cataphylla 1-хромосомасында және *Aedes vexans*, *Ae.caspius* және *Ae.subdiversus* 2-хромосомада рДНҚ локустарын анықтады.

Кілт сөздер: Aedes, қансорғыш масалар, митоздық хромосомалар, С-бояғыш, DAPI, FISH, рДНҚ, гетерохроматин.

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Сравнительный кариологический анализ четырёх видов комаров рода *Aedes*, обитающих на территории Республики Казахстан

В настоящем исследовании проведён кариотипический анализ четырёх видов комаров рода *Aedes* (*Aedes vexans*, *Ae. caspius*, *Ae. cataphylla*, *Ae. subdiversus*), собранных на территории Республики Казахстан. В ходе работы были выполнены измерения длины хромосом у каждого из исследуемых видов, а также применены методы С-окрашивания, DAPI-флуоресценции и флуоресцентной гибридизации in situ (FISH) с использованием зонда 18S рДНК. С-окраска и DAPI-флуоресценция показали, что участки гетерохроматина локализуются преимущественно в центромерных зонах хромосом. По результатам FISH-гибридизации установлено, что у *Ae. cataphylla* локусы 18S рДНК располагаются на первой хромосоме, тогда как у *Aedes vexans*, *Ae. caspius* и *Ae. subdiversus* они выявлены на второй хромосоме.

Ключевые слова: *Aedes*, кровососущие комары, митотические хромосомы, С-окраска, DAPI, FISH, rDNA, гетерохроматин.

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Genotyping of Vitamin-D Receptor (VDR) gene polymorphisms rs7975232, rs1544410, rs731236 and analysis of their association with susceptibility to SARS-CoV-2 among the Kazakh ethnic group

This pilot study investigated the single nucleotide polymorphisms rs731236, rs1544410, and rs7975232 of the VDR gene using real-time ARMS-PCR in a cohort of 119 individuals of Kazakh ethnic group. Participants were stratified into COVID-19-positive (p-COVID-19; n = 88) and COVID-19-negative (no-COVID-19; n = 31) groups based on the detection of SARS-CoV-2-specific IgM and IgG antibodies by ELISA. The allelic and genotypic distributions of all three SNPs conformed to Hardy–Weinberg equilibrium. No statistically significant differences in allele or genotype frequencies were observed between the groups for rs731236, rs7975232, or rs1544410 ($p > 0.05$), indicating that these polymorphisms do not influence susceptibility to SARS-CoV-2 infection in the studied population. A borderline association was noted for the heterozygous CT genotype of rs1544410 ($p = 0.0548$), suggesting a potential protective effect (OR = 0.426; 95 % Cl: 0.1816 — 0.9563). Despite the limited sample size, this is the first study to examine rs731236, rs1544410, and rs7975232 in relation to SARS-CoV-2 susceptibility within the Kazakh ethnic population, as well as one of the few to simultaneously analyze all four alleles of rs1544410.

Keywords: vitamin D receptor (VDR) gene, single nucleotide polymorphism, rs731236, rs7975232, rs1544410, SARS-CoV-2, COVID-19, susceptibility, Kazakh ethnic group.

Introduction

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has had a devastating impact on global health and economic systems. Marked by high transmissibility, substantial mortality, and long-term health consequences, the pandemic has revealed the limitations of current treatment strategies. Although mass vaccination campaigns and public health measures have helped curb the spread of the virus, there remains no universally effective therapy, especially in patients suffering from severe respiratory complications. This highlights the urgent need to identify biological factors that influence susceptibility to the virus. One promising area of investigation is the role of genetic variation, particularly single nucleotide polymorphisms (SNPs), which may influence individual susceptibility to infection. The vitamin D receptor (VDR) gene has emerged as a potential genetic marker of interest in this context [1, 2].

Vitamin D is known for its immunomodulatory and anti-inflammatory properties. Deficient levels of vitamin D have been associated with a higher risk of various chronic and infectious diseases, including cancers, autoimmune conditions, cardiovascular disorders, and respiratory infections [3]. Increasingly, clinical and epidemiological data suggest a strong link between low serum levels of vitamin D and an elevated risk of contracting COVID-19 [4]. This association has also been observed in individuals of Kazakh ethnicity, among whom vitamin D deficiency is relatively prevalent [5].

The biological effects of vitamin D are mediated through its active form, calcitriol, which binds to the VDR—a nuclear receptor encoded by a highly polymorphic gene located on chromosome 12. Among the numerous SNPs identified in the VDR gene, four have been widely studied for their potential role in disease susceptibility: rs228570, rs7975232, rs1544410, and rs731236. Of these, rs7975232, rs1544410, and rs731236 are positioned in the 3′ untranslated region (3′ UTR) and are in strong linkage disequilibrium, often referred to collectively as the 3′ UTR polymorphisms [6, 7].

According to dbSNP data, the rs731236 polymorphism includes three alleles, with A and G being predominant; rs7975232 comprises the A and C alleles; and rs1544410 contains all four possible allelic variants [8–10]. However, both the reported allele composition and the number of alleles analyzed for these SNPs can vary across studies. For example, some researchers investigate rs731236 in terms of the T and C alleles [11, 12], while rs1544410 is commonly analyzed using only two allelic variants at a time—either A

and G [11] or A and T [13]. Therefore, in the present study, the allele composition is considered according to the dbSNP database, and all four alleles of rs1544410 are analyzed simultaneously.

Although numerous studies have examined the association between these three VDR variants and COVID-19 susceptibility, their findings remain inconclusive and vary across different ethnic groups and methodological approaches. Given these discrepancies, the present study aims to explore the relationship between VDR polymorphisms rs731236, rs1544410, rs7975232 and susceptibility to SARS-CoV-2 infection in individuals of Kazakh ethnic group.

Experimental

A total of 119 volunteers participated in the study. Participant selection was based on a preliminary questionnaire, with key inclusion criteria being age over 18 years, no COVID-19 vaccination received within the past 12 to 18 months, and belonging to the Kazakh ethnic group. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Local Bioethics Committee of the Non-commercial Joint-Stock Company "Karaganda Medical University" (Protocol No. 2, dated 11 October 2022). Written informed consent was obtained from all participants.

Venous blood samples were collected into two EDTA tubes. One tube was centrifuged to obtain plasma, which was subsequently analyzed via enzyme-linked immunosorbent assay (ELISA) to detect SARS-CoV-2-specific IgM and IgG antibodies. The following diagnostic kits were employed: SARS-CoV-2-IgG-ELISA-BEST and SARS-CoV-2-IgM-ELISA-BEST (Vector-Best, Novosibirsk, Russia).

Previous studies have shown that IgM and IgG antibody levels typically begin rising simultaneously within the first week of SARS-CoV-2 infection [14]. IgM titers typically decline and become undetectable within approximately three months of symptom onset, whereas IgG antibodies persist, gradually decreasing over a period of 4–7 months [15, 16]. Therefore, IgM and/or IgG titers exceeding the diagnostic threshold (>1 ng/mL) were considered as evidence of an active or recently resolved SARS-CoV-2 infection (within 1 week to 6 months prior to sampling).

Based on ELISA results, participants were stratified into two groups: SARS-CoV-2-positive (p-COVID-19) and SARS-CoV-2-negative (no-COVID-19). Summary demographic and clinical characteristics of the participants by group are provided in Table 1.

Table 1

General characteristics of the study groups

	p-COVID-19	**no-COVID-19**
Total (n)	88	31
Age (years; mean ± SD)	43 ± 14.38	41 ± 15.24
Sex (M / F)	27 / 61	12 / 19
IgM (ng/mL; mean ± SD)	1.691 ± 3.008	0.399 ± 0.181
IgG (ng/mL; mean ± SD)	7.086 ± 3.881	0.3191 ± 0.2282

The second blood sample was used for genotyping the VDR gene SNPs rs731236, rs1544410, and rs7975232. Genomic DNA was isolated from whole blood using the RIBO-prep kit (AmpliSens, Moscow, Russia) following the manufacturer's instructions. DNA concentration and purity were assessed using a DS-11 spectrophotometer (DeNovix Inc., Wilmington, DE, USA). Genotyping was performed using real-time polymerase chain reaction (PCR) with forward and reverse outer and inner primers (Lumiprobe, Russia), based on the amplification-refractory mutation system (ARMS) technique.

Each 25 μL PCR reaction included 50 ng of genomic DNA, 10 pmol of each allele-specific or control primer pair (FIP–ROP, RIP–FOP, or FOP–ROP), Taq polymerase, dNTPs, and PCR buffer (GeneLab, Astana, Kazakhstan). Amplification was carried out in a DTlite real-time PCR system (DNA Technology, Moscow, Russia) using Real-Time_PCR software v.7.9 (DNA Technology). Primer sequences and PCR cycling parameters are provided in Figure.

SNP	Alleles	Primer sequence	PCR protocol
rs731236	A G	FIP 5'-CGGTCCTGGATGGCCGCA-3' RIP 5'-CAGGACGCCGCGCTGCTC-3' FOP 5'-TTGGCATAGAGCAGGTGGCTGCC-3' ROP 5'-CCCAGCTGAGAGCTCCTGTGCCTT-3'	94°C / 3 min (94°C / 15 sec, 62°C / 30 sec) × 40
rs7975232	A C	FIP 5'-CACAGGAGCTCTCAGCTGGACA-3' RIP 5'-TGGTGGGATTGAGCAGTGAAGG-3' FOP 5'-CCTGGATGGCCTCAATCAGC-3' ROP 5'-GTCATAGAGGGGTGGCCTAGGG-3'	94°C / 3 min (94°C / 15 sec, 62°C / 30 sec) × 40
rs1544410	C A	FIP 5'-CAGAGCCTGAGTATTGGGAACGC-3' RIP 5'-GGGGCCACAGACAGGCCTACT-3' FOP 5'-TTTTGTACCCTGCCCGCAAGA-3' ROP 5'-TGTGCAGGCGATTCGTAGGG-3'	
	C G	FIP 5'-AGCAGAGCCTGAGTATTGGGAAAGC-3' RIP 5'-GGCCACAGACAGGCCTCCC-3' FOP 5'-AAGTTTTGTACCCTGCCCGCAAG-3' ROP 5'-GTGCAGGCGATTCGTAGGGG-3'	94°C / 3 min (94°C / 15 sec, 62°C / 30 sec) × 40
	T C	FIP 5'-GCAGAGCCTGAGTATTGGGAAGGT-3' RIP 5'-GGCCACAGACAGGCCTTCG-3' FOP 5'-AAGTTTTGTACCCTGCCCGCAA-3' ROP 5'-TGTGCAGGCGATTCGTAGGG-3'	

Figure. The designed primer sequences and thermal cycling conditions for genotyping rs731236, rs7975232, and rs1544410 using the ARMS-PCR

Following real-time amplification, allele-specific reactions were subjected to melting curve analysis under the following protocol: 15 seconds at 90 °C followed by 100 cycles with 0.5 °C increments.

A complete description of the genotyping methodology for rs731236, rs1544410, and rs7975232 is provided in the methodological guidelines [17].

Continuous variables were expressed as mean ± standard deviation (SD). Categorical variables were reported as percentages and compared using the chi-square or Fisher's exact test where appropriate. Hardy–Weinberg equilibrium (HWE) was assessed using chi-square distribution. Odds ratios (ORs) with 95 % confidence intervals (CIs) were calculated. Two-tailed p-values < 0.05 were considered statistically significant. Statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA).

Results and Discussion

To evaluate whether the VDR gene polymorphisms rs731236, rs1544410, and rs7975232 influence susceptibility to SARS-CoV-2 infection, we compared allele and genotype frequencies for each SNP between COVID-19-positive and COVID-19-negative groups. All genotype distributions were in Hardy–Weinberg equilibrium (HWE), with p-values > 0.05: p = 0.0628 for rs731236, p = 0.1125 for rs7975232, and p = 0.5381 for rs1544410. Genotyping results and comparisons for these VDR SNPs are presented in Table 2.

Table 2

Allele and genotype frequencies of rs731236, rs1544410, and rs7975232 within the studied groups

	p-COVID-19 (n)	**no-COVID-19 (n)**	**OR (CI 95 %)**	χ^2	**p-value#**
1	**2**	**3**	**4**	**5**	**6**
rs731236					
Alleles	n = 176 (Freq.)	n = 62 (Freq.)			
A	115 (65.34 %)	41 (66.13 %)	0.9656 (0.5176 — 1.772)	0.01261	0.9106
G	61 (34.66 %)	21 (33.87 %)	1.036 (0.5643 — 1.932)	0.01261	0.9106

Continuation of Table 2

1	2	3	4	5	6
Genotypes	n = 88 (Freq.)	n = 31(Freq.)			
AA	47 (53.41 %)	18 (58.06 %)	0.8279 (0.3520 — 1.822)	0.2004	0.6807
AG	21 (23.86 %)	5 (16.13 %)	0.63 (0.5898 — 4.282)	0.8032	0.4548
GG	20 (22.73 %)	8 (25.81 %)	0.8456 (0.3198 — 2.179)	0.1208	0.8064
rs7975232					
Alleles	n = 176 (Freq.)	n = 62 (Freq.)			
A	77 (43.75 %)	25 (40.32 %)	1.151 (0.6481 — 2.046)	0.2199	0.6576
C	99 (56.25 %)	37 (59.68 %)	0.8687 (0.4888 — 1.543)	0.2199	0.6576
Genotypes	n = 88 (Freq.)	n = 31(Freq.)			
AA	20 (22.73 %)	6 (19.35 %)	1.225 (0.4706 — 3.401)	0.1527	0.8039
AC	37 (42.05 %)	13 (41.94 %)	1.005 (0.4542 — 2.372)	0.0001138	0.9915
CC	31 (35.63 %)	12 (38.71 %)	0.8765 (0.3878 — 1.954)	0.09346	0.8291
rs1544410					
Alleles	n = 176 (Freq.)	n = 62 (Freq.)			
C	120 (68.18 %)	43 (69.35 %)	0.9468 (0.5175 — 1.779)	0.02923	0.8642
G	14 (7.95 %)	2 (3.23 %)	2.593 (0.6581 — 11.74)	1.635	0.2514
T	42 (23.86 %)	17 (27.42 %)	0.8397 (0.4380 — 1.635)	0.3109	0.6095
Genotypes	n = 88 (Freq.)	n = 31(Freq.)			
CC	40 (45.45 %)	12 (38.71 %)	1.319 (0.5964 — 2.934)	0.4239	0.5363
TT	5 (5.68 %)	NA	NA	1.839	0.3248
GG	1 (1.14 %)	NA	NA	0.3553	0.5512
CT	30 (34.09 %)	17 (54.84 %)	0.426 (0.1816 — 0.9563)	4.13	0.0548
GT	2 (2.27 %)	NA	NA	0.7166	0.3973
CG	10 (11.36 %)	2 (6.45 %)	1.859 (0.4541 — 8.861)	0.61	0.7293

[#]p-values were calculated using Fisher's exact test
$p > 0.05$ = not significant.
Abbreviation: NA, not available

As shown in Table 2, no statistically significant differences were found in allele or genotype frequencies between the p-COVID-19 and no-COVID-19 groups for any of the three 3′ UTR polymorphisms.

In the case of rs731236, allele A was present in 65.34 % of the p-COVID-19 group and 66.13 % of no-COVID-19, while allele G occurred in 34.66 % and 33.87 %, respectively. The odds ratio (OR) for alleles A and G were 0.9656 (95 % CI: 0.5176–1.772; $p = 0.9106$) and 1.036 (95 % CI: 0.5643–1.932; $p = 0.9106$), indicating no significant association with infection risk.

The genotypic analysis showed that genotype AA was present in 53.41 % of COVID-19-positive participants and 58.06 % of COVID-19-negative ones, yielding an OR = 0.8279 (95 % CI: 0.3520–1.822), $p = 0.6807$. Heterozygous genotype AG occurred in 23.86 % of p-COVID-19 versus 16.13 % in no-COVID-19 (OR = 0.63, $p = 0.4548$), while homozygous GG was found in 22.73 % and 25.81 % respectively (OR = 0.8456, $p = 0.8064$). None of these comparisons reached statistical significance, indicating that rs731236 genotypes do not appear to be associated with susceptibility to SARS-CoV-2 infection in this sample.

For rs7975232, the A allele appeared in 43.75 % of COVID-19-positive individuals and 40.32 % of COVID-19-negative ones. The C allele was slightly more prevalent in both groups (56.25 % and 59.68 %, respectively). The OR for allele A was 1.151 (95 % CI: 0.6481–2.046; $p = 0.6576$).

Genotype frequencies were also comparable. The AA genotype was observed in 22.73 % of COVID-19-positive individuals and 19.35 % in no-COVID-19 group (OR = 1.225, $p = 0.8039$). The AC genotype was almost equally represented (42.05 % in cases vs. 41.94 % in controls; OR = 1.005, $p = 0.9915$), and CC occurred in 35.63 % and 38.71 % of the respective groups (OR = 0.8765, $p = 0.8291$).

These findings are consistent with results reported by Jafarpoor et al. in an Iranian cohort, where no association was found between rs731236 or rs7975232 and COVID-19 susceptibility [18]. An ecological study involving data from 26 countries did report a positive correlation between the frequency of the rs731236 TT genotype and COVID-19 prevalence ($r = 0.42$, $p = 0.03$), as well as between the rs7975232 AA genotype and both COVID-19 prevalence ($r = 0.45$, $p = 0.02$) and mortality ($r = 0.42$, $p = 0.03$) [12]. However, methodological differences and population heterogeneity preclude direct comparison with our data.

Analysis of rs1544410 allele frequencies also revealed no significant differences between groups. The C allele was most prevalent across both groups, with 68.18 % in p-COVID-19 and 69.35 % in no-COVID-19 (OR = 0.9468, p = 0.8642). The T allele was present in 23.86 % and 27.42 %, respectively (OR = 0.8397, p = 0.6095), while the G allele occurred in only 7.95 % of COVID-19-positive participants and 3.23 % of COVID-19-negative (OR = 2.593, 95 % CI: 0.6581–11.74, p = 0.2514), indicating a non-significant trend toward higher G allele frequency in p-COVID-19 group.

Importantly, allele A at the rs1544410 locus was absent in all participants, consistent with the known allele distribution in the Kazakh ethnic group, possibly due to regional or ethnic-specific genetic architecture.

Among genotypes, CC was observed in 45.45 % of COVID-19-positive subjects and 38.71 % of COVID-19-negative (OR = 1.319, p = 0.5363). The heterozygous CT genotype was less frequent in p-COVID-19 group (34.09 %) compared to no-COVID-19 group (54.84 %), yielding a result at the threshold of statistical significance (OR = 0.426, 95 % CI: 0.1816–0.9563, p = 0.0548). This may suggest a potential protective effect of this genotype, although it narrowly missed the conventional threshold for statistical significance.

The TT genotype was found in 5.68 % of COVID-19-positive participants and was absent in no-COVID-19 group, precluding calculation of a reliable odds ratio (χ^2 = 1.839, p = 0.3248). Several genotypes—such as GG, GT, and CG—were detected at very low frequencies in our sample. This limited occurrence reduces statistical power and makes it challenging to draw meaningful conclusions about their association with SARS-CoV-2 susceptibility. As a result, any observed trends involving these rare variants should be interpreted with caution and considered exploratory rather than confirmatory.

Thus, despite some numeric variation, rs1544410 did not show a statistically significant association with SARS-CoV-2 susceptibility. Similar findings, despite methodological and population differences, were reported in the ecological study by Karcıoğlu et al. [12].

More data are available regarding the association of 3′ UTR polymorphisms with COVID-19 severity and mortality than with infection risk. However, these studies vary considerably in methodology, populations, and outcomes. For example, a 2024 systematic review encompassing 12 studies found that the rs7975232 AA and rs731236 TT genotypes were associated with increased risk of COVID-19-related death. Additionally, rs1544410 may serve as a predictive biomarker for disease severity, while all three polymorphisms were considered potential markers of mortality risk [19]. In contrast, a study by Tentolouris et al. in a Caucasian Greek cohort found no association between rs7975232, rs731236, and COVID-19 severity [20] and Saba et al. found that in the recessive model, the T/T rs7975232 genotype was statistically associated with a lower risk of the infection severity [7].

We acknowledge that limitations of the current work include a relatively small sample size, which may have reduced the statistical power to detect modest genotype–phenotype associations. This limitation increases the risk of errors, particularly in the analysis of rare genotypes such as GG, GT, and CG, which were underrepresented in our cohort.

Despite these limitations, the study possesses several notable strengths. To our knowledge, this is the first investigation of VDR gene polymorphisms and SARS-CoV-2 susceptibility conducted specifically within the Kazakh ethnic population. Primer combinations were also developed for the ARMS-PCR technique to allow simultaneous analysis of all four allelic variants of the rs1544410 polymorphism.

Although our study in Kazakh individuals did not find a significant association between rs731236, rs1544410, or rs7975232 and COVID-19 susceptibility, inconsistent results across populations and study designs highlight the need for further investigation of the role of VDR gene polymorphisms in SARS-CoV-2 infection, including larger multi-ethnic cohorts to improve generalizability and statistical robustness.

Conclusions

This pilot study was conducted among 119 individuals of Kazakh ethnic origin, who were stratified into COVID-19-positive and COVID-19-negative groups based on ELISA testing for the presence or absence of antibodies against SARS-CoV-2. Genotyping of the VDR gene single nucleotide polymorphisms rs731236, rs1544410, and rs7975232, followed by a comparative analysis of allele and genotype frequencies between the groups, revealed no statistically significant differences.

Based on these findings, it can be generally concluded that the rs731236, rs1544410, and rs7975232 polymorphisms do not appear to influence susceptibility to COVID-19 within the Kazakh population (p > 0.05). However, the observation of borderline statistical significance for the rs1544410 CT genotype,

the relatively small sample size, the limited number of peer-reviewed studies on this topic, and the conflicting results reported in the literature underscore the need for further research in this area.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Protas V.V.** – Conceptualization, Data curation, Investigation, Writing draft; **Pogossyan G.P.** – Supervision, Conceptualization, Data curation, Formal analysis, Writing – review and editing; **Li K.G.** – Methodology, Supervision, Conceptualization, Formal analysis; **Zhumina A.G.** – Formal analysis, Writing – review and editing; **Bisseneva A.K.** – Investigation, Data curation, Editing.

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Қазақ этникалық тобындағы SARS-CoV-2-ге бейімділікпен байланыстағы витамин D рецепторы (VDR) генінің rs7975232, rs1544410 және rs731236 полиморфизмдерін генотиптеу және олардың байланысын талдау

Бұл пилоттық зерттеуде қазақ этникалық тобына жататын 119 адамның үлгісінде VDR генінің rs731236, rs1544410 және rs7975232 бір нуклеотидті полиморфизмдері нақты уақытта ARMS-ПЦР әдісімен зерттелді. Қатысушылар SARS-CoV-2-ге спецификалық IgM және IgG антиденелерінің ИФА арқылы анықталуына байланысты COVID-19-оң (p-COVID-19; n = 88) және COVID-19-теріс (no-COVID-19; n = 31) топтарға бөлінді. Үш SNP бойынша аллельдік және генотиптік таралу Харди–Вайнберг тепе-теңдігіне сәйкес келді. rs731236, rs7975232 және rs1544410 үшін топтар арасында аллельдер мен генотиптер жиіліктерінде статистикалық тұрғыдан маңызды айырмашылықтар анықталған жоқ (p > 0.05), бұл осы полиморфизмдердің зерттеліп отырған популяцияда SARS-CoV-2 инфекциясына бейімділікке әсер етпейтінін көрсетеді. rs1544410-ның гетерозиготалы CT генотипі үшін шекті маңыздылықтағы байланыс байқалды (p = 0.0548), бұл генотиптің мүмкін болатын қорғаныштық әсерін ұсынады (OR = 0.426; 95 % Cl: 0.1816 — 0.9563). Үлгінің көлемі шектеулі болғанына қарамастан, бұл — қазақ этникалық тобында rs731236, rs1544410 және rs7975232 полиморфизмдерінің SARS-CoV-2-ге бейімділікпен байланысын зерттеген алғашқы жұмыс, сондай-ақ rs1544410 полиморфизмінің барлық төрт аллелін бір мезгілде талдаған сирек зерттеулердің бірі.

Кілт сөздер: D дәрумені рецепторы (VDR) гені, бір нуклеотидті полиморфизм, rs731236, rs7975232, rs1544410, SARS-CoV-2, COVID-19, бейімділік, қазақ этникалық тобы.

В.В. Протас, Г.П. Погосян, К.Г. Ли, А.Г. Жумина, А.К. Бисенева

Генотипирование полиморфизмов гена рецептора витамина D (VDR) rs7975232, rs1544410, rs731236 и анализ их связи с восприимчивостью к SARS-CoV-2 среди представителей казахской этнической группы

В настоящем пилотном исследовании проведено генотипирование однонуклеотидных полиморфизмов rs731236, rs1544410 и rs7975232 гена рецептора витамина D (VDR) методом ARMS-ПЦР в реальном времени среди 119 представителей казахской этнической группы. Участники были разделены на две группы: COVID-19-положительную (p-COVID-19; n = 88) и COVID-19-отрицательную (no-COVID-19; n = 31) на основании наличия или отсутствия специфических антител IgM и IgG к SARS-CoV-2, выявленных методом иммуноферментного анализа (ИФА). Распределение аллелей и генотипов всех трех полиморфизмов соответствовало закону Харди–Вайнберга. По результатам исследования не было выявлено статистически значимых различий в частотах аллелей и генотипов между группами для rs731236, rs7975232 и rs1544410 (p > 0,05), что позволяет предположить отсутствие их влияния на восприимчивость организма к инфицированию SARS-CoV-2 в исследуемой популяции. При этом для гетерозиготного генотипа CT полиморфизма rs1544410 зафиксирована тенденция к ассоциации с инфицированием на границе статистической значимости (p = 0,0548), что может свидетельствовать о возможном защитном эффекте (OR = 0,426; 95 % Cl: 0.1816–0,9563). Несмотря на ограниченный объем выборки, это первое исследование, посвященное анализу связи rs731236, rs1544410, rs7975232 с

восприимчивостью к SARS-CoV-2 среди представителей казахской этнической группы, а также одно из немногих, в котором проведено одновременное генотипирование всех четырех аллелей rs1544410.

Ключевые слова: ген рецептора витамина D (VDR), однонуклеотидный полиморфизм, rs731236, rs7975232, rs1544410, SARS-CoV-2, COVID-19, восприимчивость, казахская этническая группа.

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Study of adaptive states and variability of phenological development of introduced woody plants of the genus Berberis L. in the conditions of the Altai Botanical Garden

The article considers the features of phenological development and adaptive capabilities of the genus *Berberis* L. during primary introduction in the conditions of the Altai Botanical Garden. The characteristics of the origin of the collection taxa of the genus are given; the taxonomic composition of the collection fund of the genus *Berberis* L. as of 2025 is verified. The introduced species are divided by soil-geographical origin, where the introduced species are distributed into 5 groups. An introduction assessment of the genus *Berberis* L. is given based on ecological-geographical, morpho-biological characteristics. The adaptive potential of introduced species is assessed based on long-term phenological observations, where the full development cycle is established. Based on the results of the assessment of the prospects of introduced species, two groups of species prospects were identified: promising (83%) of the collection fund and less promising (17%). Based on this assessment, introduced species are recommended for use in phytomelioration, gardening and park construction, as ornamental and fruit crops in Eastern Kazakhstan.

Keywords: introduction, collection fund, genus *Berberis* L., prospects, adaptation, phenological phases.

Introduction

Currently, urbanization of cities is a significant cause of depletion of natural resources, leading to disruption of the ecological balance of plant communities. In this regard, the problem of greening recreational areas and restoring urban ecosystems under the influence of anthropogenic and technogenic activities is acute. The range of such plants is based on their resistance to aggressive environmental conditions and a complex of protective and environment-forming functions.

Plants of the genus *Berberis* L. are universal for landscaping city streets, parks, squares. Numerous representatives of the genus combine decorative, valuable food, medicinal qualities [1]. They are often used to form hedges or borders, creating natural dividing strips and adding a visual accent in green spaces, they can also be planted individually on lawns or combined into decorative groups [2]. These plants are easy to care for, while they have high decorative qualities. The bright berries of the bush attract birds and animals.

The first botanical taxonomy of the genus was compiled at the end of the 16th century by K. Linnaeus and included 2 species—*Berberis vulgaris* L. and *Berberis cretica* L. By the end of the 19th century, the genus already had 150 species. In 1905, the Austrian botanist S.K. Schneider published a monograph with the most complete description of the genus, which included 156 species from 22 sections [3]. According to the research conducted by A. Redder (1949), the genus includes 175 species [4]. According to the latest data from the website The Plant List [5], the genus has about 580 species. Plants of the genus Berberis L. belong to the department MAGNOLIOPHYTA, class MAGNOLIOPSIDA, order BERBERIDALES, family BERBERIDACEAE Juss.

In nature, they are represented by shrubs or small trees with loose, thorny branches and beautiful flowers. Wide geographical distribution of the genus *Berberis* L. (tropical, subtropical, temperate and cold zones) indicates its ancient origin. Paleobotanists note leaf imprints in layers dating back to the Oligocene and Miocene periods of Western Europe and in the Sarmatian deposits of Krynka. Modern centers of species diversity of the genus are located in Southeast Asia (China, the provinces of Sichuan, Yunnan and Southeast Tibet), in Central Asia (the Himalayas) and on the West Coast of South America [6]. The unpretentiousness of the genus and its ability to adapt allowed it to occupy vast territories not only in latitudinal zonality, but also in vertical zonality. Some species grow at an altitude of 4,300 m above sea level (*Berberis diaphana* Maxim.), are mainly deciduous shrubs from Central Asia [2].

The genus *Berberis* L. in the wild flora of Eastern Kazakhstan is represented by two species—*Berberis sibirica* Pall. and *Berberis heteropoda* Schrenk [7, 8]. Expansion of the range of plants for landscaping the cities of the East Kazakhstan region is possible with the involvement of non-regional species.

The aim of the study is to investigate the phenological characteristics and adaptive capabilities of the genus *Berberis* L. under introduction conditions.

Research objectives:

- to verify the taxonomic composition of introduced species of the collection fund of the genus *Berberis* L. in the Altai Botanical Garden;
- to establish the ecological and geographical confinement of the natural growth of introduced species of the genus *Berberis* L.;
- to provide a morphological and biological assessment of the genus under the conditions of introduction;
- to determine the calendar dates of the onset of phenological phases of development of introduced species of the genus for practical application;
- to summarize the results of the initial testing of the genus *Berberis* L. in the Altai Botanical Garden, to assess the prospects of the species for economic use.

In general, representatives of the genus Berberis L. are an important and versatile resource that finds application in various areas of human activity: in medicine, food industry and landscape design.

Objects and methods of research

The object of the research is a collection of the genus *Berberis* L. of the Altai Botanical Garden in the amount of 30 taxa, of which 20 are foreign species, 8 varieties, and 2 local species.

The climate of the introduction area is sharply continental, with characteristic frosty long winters and cool short summers. The mountainous relief softens the sharply continental climate. The average temperature in January is -12.6^0 C, the absolute maximum is 41.6^0 C. The period of active vegetation of plants is short, 69–135 days. The Selyaninov hydrothermal coefficient is within 1.2. Sufficient precipitation with average daily temperatures above 10^0 C during the growing season allows plants to adapt to a temperate climate. The soils of the arboretum are represented by mountain chernozems with a humus content of 4–6%, with a well-defined profile. Along the left edge of the site there is an oxbow lake of the Bystrukha River, which dilutes the chernozems with sandy soils and provides additional soil drainage [9].

Phenological observations of introduced species were carried out according to the Methodology of Phenological Observations in Botanical Gardens of the USSR (1979) [10, 11].

Biometric analysis of shoots was performed according to the method of G.F. Lakin (1990) [12].

Evaluation of the viability and prospects of introduced species based on visual observations using the scale of P.I. Lapin (1973) and M.N. Kosaev (1987) [13, 14].

Evaluation of the introduction value of species of the genus *Berberis* L. will allow to expand the range of products for economic use in Eastern Kazakhstan [15, 16].

Research results and their discussion

Testing of the genus *Berberis* in the Altai Botanical Garden began in 1952. The first taxa were grown from seeds of unknown origin and were represented by species specimens of *B. vulgaris* L., *B. integerrima* Bunge, *Berberis x serrata* Koehne. The species were selected based on the ecological conditions of plant growth in natural conditions. About 35 species, forms and varieties were tested during this period. The samples of taxa currently in the collection were grown from seeds of cultural origin obtained from countries of the near and far abroad. The collection includes plants obtained from seeds of their own reproduction and natural populations.

The varietal material was mainly drawn from a private nursery located in the outskirts of Almaty, using live plants. Currently, the collection of the Altai Botanical Garden contains 30 species, forms and varieties of the genus *Berberis* L., which are adapted to varying degrees to the harsh climatic conditions of the Kazakh Altai.

The characteristics of the origin of the collection plants are given in Table 1.

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Table 1

Characteristics of the origin of collection taxa of the genus Berberis L.

Species name	Origin of source material/age
B. × *ottawensis* Schneid. cv. *Superba*	Russia, NIISS named after Lisavenko, Barnaul, live plants/2007
B. amurensis Rupr.	Russia, Moscow, VILR, seeds/1979
B. chinensis Poir.	Ireland, Dublin, National Botanic Garden, seeds/2005
B. circumserrata (C.K. Schneid.) C.K. Schneid..	Russia, Moscow, GBS, seeds/1985
B. crataegina DC.	Belgium, Beveren, Municipal Arboretum, seeds/2007
B. diaphana Maxim.	Ireland, Dublin, National Botanic Garden, seeds/2004
B. dielsiana Fedde	Russia, Ussuri region, Mountain Taiga station, seeds/2008
B. heteropoda Schrenk	Kazakhstan, ABS, reproduction, seeds/2008
B. integerrima Bunge	Unknown/1955
B. koreana Palib.	Russia, NIISS named after Lisavenko, Barnaul, live plants/1970
B. lecomtei Schneid.	Sweden, Bergian Botanical Garden, Stockholm, seeds/1972
B. orientalis Schneid.	Russia, Stavropol Botanical Garden named after V.V. Skripchinsky, Stavropol, seeds/1966
B. poiretii C.K. Schneid.	Kazakhstan, GBS Almaty, seeds/1989
B. sibirica Pall.	Kazakhstan, East Kazakhstan region, Listvyaga ridge, seeds of natural origin/1991
B. sieboldii Mig.	Belarus, Centralized Botanical Garden, Minsk, seeds/1981
B. silva-taroucana Schneid.	Lithuania, Kaunas Botanical Garden, Kaunas, seeds/1977
B. thunbergii DC.	Russia, NIISS named after Lisavenko, Barnaul, live plants/2002
B. th. cv. *Carmen*	Kazakhstan, Almaty, nursery “Mountain gardener”, live plants/2007
B. th. cv. *Atropurpurea Nana*	Kazakhstan, Almaty, nursery “Mountain gardener”, live plants/2007
B. th. cv. *Colden Ring*	Kazakhstan, Almaty, nursery “Mountain gardener”, live plants/2009
B. th. cv. *Kornik*	Kazakhstan, Almaty, nursery “Mountain gardener”, live plants/2007
B. th. cv. *Maria*	Kazakhstan, Almaty, nursery “Mountain gardener”, live plants/2009
B. th. cv. *Pink Attraction*	Kazakhstan, Almaty, nursery “Mountain gardener”, live plants/2007
B. th. cv. *Red Rocket*	Kazakhstan, Almaty, nursery “Mountain gardener”, live plants/2009
B.th. f. *atropurpurea* Chenault	Germany, Botanical Garden of the University of Mainz/2002
B. turcomanica Karel.	Italy, Botanical Garden of the Technical University of Udine, seeds/2007
B. vulgaris f. *atropurpurea* Bunge	Russia, TSHA Moscow, seeds/1971
B. vernae Schneid.	Germany, Botanical Garden of Berlin, seeds/1969
B. virescens Hook.	Russia, Botanical Garden of the Ural Branch of the Russian Academy of Sciences, Sverdlovsk, seeds/1976
B. vulgaris L.	Kazakhstan, ABS, reproduction, seeds/2009

Taxonomic composition of the collection fund of the genus *Berberis* L. is represented by 8 sections, 3 subsections (Table 2):

Table 2

Taxonomic composition of the collection fund of the Altai Botanical Garden of the genus *Berberis* L.

Section	Subsection	Name of species
Vulgares		*Berberis vulgaris* L.
		Berberis vulgaris f. atropurpurea Bunge
		Berberis sieboldii Mig.
		Berberis orientalis Schneid.
		Berberis koreana Palib.
		Berberis amurensis Rupr.
Sinenses		*Berberis chinensis* Poir.
		Berberis poiretii C.K. Schneid.

Continuation of Table 2

Section	Subsection	Name of species
Angulosae	Diaphanous	*Berberis diaphana* Maxim.
		Berberis circumserrata Schneid.
		Berberis virescens Hook.
	Eufranchetime	*Berberis. lecomtei* Schneid.
	Siberian	*Berberis sibirica* Pall.
Dasystach		*Berberis dielsiana* Fedde
Integerrimae		*Berberis integerrima* Bunge
		Berberis turcomannica Karel.
		Berberis vernae Schneid.
Tschonoskyanae		*Berberis* × *ottawensis Schneid.* cv. *Superba*
		Berberis thunbergii DC.
		Berberis thunbergii f. atropurpurea Chenault
		Berberis thunbergii cv. *Atropurpurea Nana*
		Berberis thunbergii cv. *Carmen*
		Berberis thunbergii cv. *Colden Ring*
		Berberis thunbergii cv. *Maria*
		Berberis thunbergii cv. *Kornik*
		Berberis thunbergii cv. *Red Rocket*
		Berberis thunbergii cv. *Pink Attraction*
		Berberis silva- taroucana Schneid.
Heteropodae		*Berberis heteropoda* Schrenk
Crataeginae		*Berberis crataegina* DC.

Territorially, according to soil-geographical origin, introduced species are classified into 5 regions: North American, East Asian, Central Asian, Central Asian—Kazakhstan and European-Caucasian [2] (Table 3).

Table 3

Territorial division by soil-geographical origin of the collection fund of ABS *Berberis* L.

Types/areas	European-Caucasian	Central Asian - Kazakhstan	Central Asian	East Asian	North American
1	2	3	4	5	6
Berberis amurensis Rupr.				+	
B. chinensis Poir.	+				
B. circum — serrata Schneid.				+	
B. crataegina DC.	+				
B. diaphan Maxim.			+		
B. dielsiana Fedde			+		
B. integerrima Bunge		+			
B. koreana Palib.				+	
B. lecomtei Schneid.			+		
B. orientalis Schneid.	+				
B. × ottawensis Schneid. cv. *Superba*					+
B. poiretii C.K. Schneid.				+	
B. sieboldii Mig.				+	
B. sibirica Pall.		+			
B. silva — taroucana Schneid.			+		
B. heteropoda Schrenk		+			
B. thunbergii DC.				+	
B. thunbergii. f. atropurpurea Chenault				+	
B. turcomanica Karel.		+			
B. vernae Schneid.			+		

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Continuation of Table 3

1	2	3	4	5	6
B. virescens Hook.					+
B. vulgaris L.	+				
B. vulgaris f. atropurpurea Bunge	+				
B. thunbergii cv. *atropurpurea nana*					
B. thunbergii cv. *Carmen*					
B. thunbergii cv. *Colden Ring*					
B. thunbergii cv. *Maria*					
B. thunbergii cv. *Kornik*					
B. thunbergii cv. *Red Rocket*					
B. thunbergii cv. *Pink Attraction*					

As of 2025, the collection of the genus *Berberis* L. is dominated by introduced species from the East Asian and Central Asian regions, which account for 29% of the Altai Botanical Garden's collection. Species from the European-Caucasian region account for 12.5%, while those from the Central Asian-Kazakhstan region make up 16%. The North American region is represented by a single species—*B.* × *ottawensis* Schneid. cv. *Superba*.

The introduction assessment of the genus *Berberis* includes an analysis of potential risks and benefits associated with the introduction of its species into new regions. The assessment consists of the following characteristics of the introduced species: eco-geographical, morpho-biological, adaptive. The history of the development of species in certain soil-geographical conditions determines their ecology.

In relation to soil fertility, the introduced species of the genus *Berberis* L. are oligotrophs—17 (57%), mainly natives of the Central Asian and East Asian regions. Mesotrophs are represented by 6 (20%) species—natives of the East Asian region—*B. amurensis*, *B. koreana*, *B. poirretii*, *B. sieboldii*, Central Asian—*B. dilsiana*, Central Asian-Kazakhstan—*B. integerrima* and make up a smaller part of the collection. Eutrophs are represented by the European—Caucasian and North American races 7 (23%) (Fig. 1).

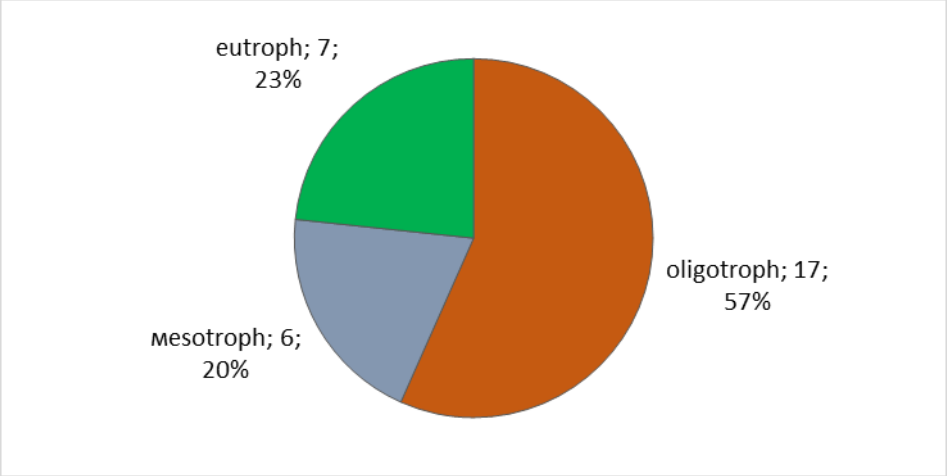

Figure 1. Classification of the collection of the genus *Berberis* L. in relation to soil fertility

The largest percentage of collection species of barberries are xerophytes from East and Central Asia—9 (30%) and mesoxerophytic species—natives of mountainous regions, which make up 13 (43%). The European-Caucasian race in the collection fund of barberries is represented by mesophytes—8 (27%). Plants of this genus tolerate waterlogging, while thickening of shoots and an increase in the rate of shoot growth are noted, which is not always a positive condition for introduction (Fig. 2) [16].

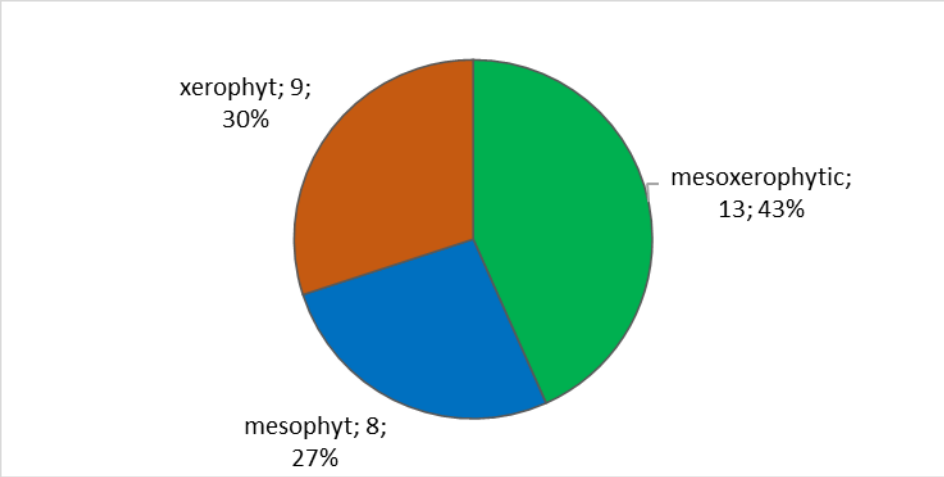

Figure 2. Classification of the collection of the genus *Berberis* L. by moisture conditions

The genus *Berberis* L. is characterized by high photophilousness, although many species are also capable of growing in partial shade. Representatives of the European-Caucasian and East Asian regions tolerate shading satisfactorily in conditions of a sharply continental climate.

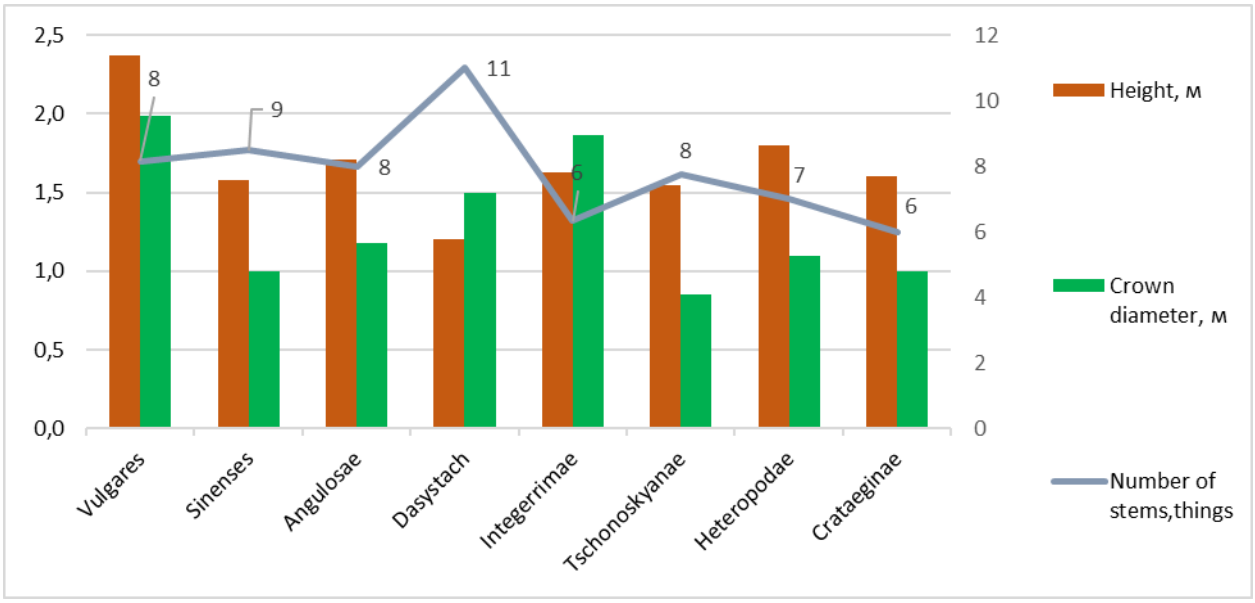

Figure 3. Changes in biological characteristics of introduced species of the genus *Berberis* L. in the arboretum

Changes in the biological characteristics of introduced species clearly reflect their adaptive capacity to the sharply continental conditions of the introduction site and are evident to varying degrees in traits such as height, crown structure, shoot formation, and reproductive ability (Fig. 3).

All species show a decrease in bush height by 20 to 35%, the crown diameter is preserved due to the good shoot-forming ability of the species. Under the conditions of introduction, obvious changes in biological parameters are observed in the Integerrimae section, where under the conditions of introduction, the height of the bushes decreases by 40% while maintaining the crown diameter. Skeletal branches are strong, thick at the base, from 5 to 8 pcs. The Sinenses section is represented by 2 species, one of which (*B. poiretii*) has good adaptation to the harsh conditions of Eastern Kazakhstan, adapts with difficulty *B. chinensis* to changes in environmental conditions, which is expressed in a decrease in the height of the bush by 35% compared to the genetically determined height, a weakening of the shoot-forming ability and a decrease in the number of stems to 5–7 pieces, while in natural growing conditions their number is 12–20 pieces. In the species from the Angulosae section, under the conditions of introduction, a slight increase in biometric indi-

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cators is noted—in height by 5–10%, all bushes with well-developed stems from 5 to 12 pieces. The Vulgares section is distinguished by high plasticity of species to environmental conditions of a sharply continental climate, preserving the habit of the bush. Introduced species from other sections showed minor changes in biometric indicators [17].

The annual average growth rates in height and trunk diameter of introduced species indicate the degree of their adaptability to unfavourable environmental conditions (Fig. 4).

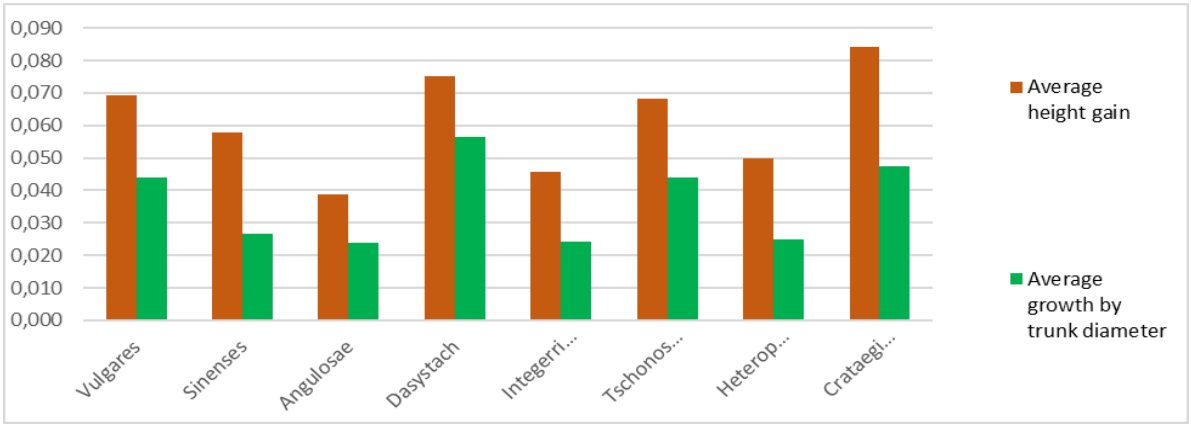

Figure 4. Indicators of annual average growth in height and trunk diameter of introduced species of the genus Berberis L., m

The most pronounced adaptive abilities for recovery after the winter period were demonstrated by species belonging to the sections Vulgares, Dasystach, Tschonoskyanae, Crataeginae. According to long-term indicators, the annual growth of species of this group fluctuates from 0.068 to 0.084 m in height and from 0.044 to 0.056 m in trunk diameter. Species of the sections Angulosae and Integerrimae are restored annually, but with lower indicators from 0.039-0.046 m and 0.024 m, respectively.

The most significant indicator influencing the adaptive processes of plants under introduction conditions is the ability to withstand prolonged, stable frosts in the winter period, early autumn frosts and the return of late spring frosts [18].

An analysis of the nature of overwintering of species based on long-term observations has established that the majority of species have winter hardiness of grade II, with partial damage to the annual growth (shoot) (Fig. 5). Average winter hardiness of grade III with complete damage to the annual growth is noted in the Sinenses and Angulosae sections.

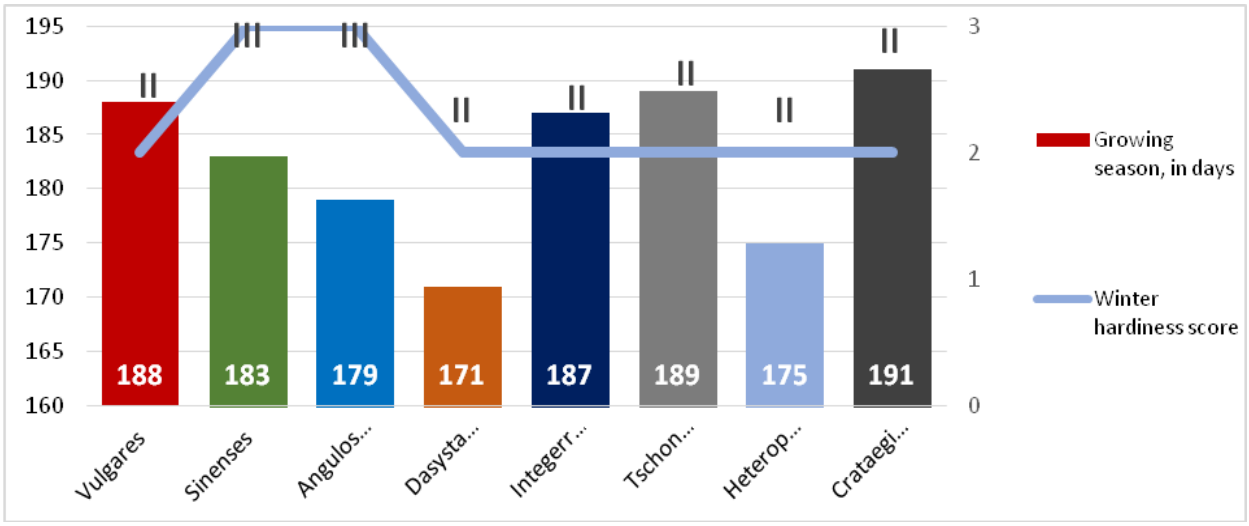

Figure 5. Indicators of winter hardiness and growing season of introduced species of the genus *Berberis* L.

High winter hardiness—I point is noted in introduced species from the Central Asian-Kazakhstan and East Asian regions. Damage to perennial lignified shoots (IV point of winter hardiness) is noted in barberries from the Angulosae (*B. lecomtei*) and Sinenses (*B. chinensis*) sections.

Comparison of the average degree of potential winter hardiness and the duration of the growing season by sections showed that all sections are characterized by an extended growing season, and an increase in the duration of which does not have a decisive effect on the degree of winter hardiness (Fig. 5). The maximum growing season is in the Crataeginae section and is 191 days, the minimum growing season is typical for the Dasystach section—171 days. Reduced winter hardiness (III points) is noted in the Sinenses and Angulosae sections. Weak winter hardiness is determined by the genotype of the introduced species, which come from the temperate zone of the Northern Hemisphere.

In new ecological conditions, introduced species adapt by changing the timing of phenological development phases, which also affects the preparation of plants for overwintering (Fig. 6) [19, 20].

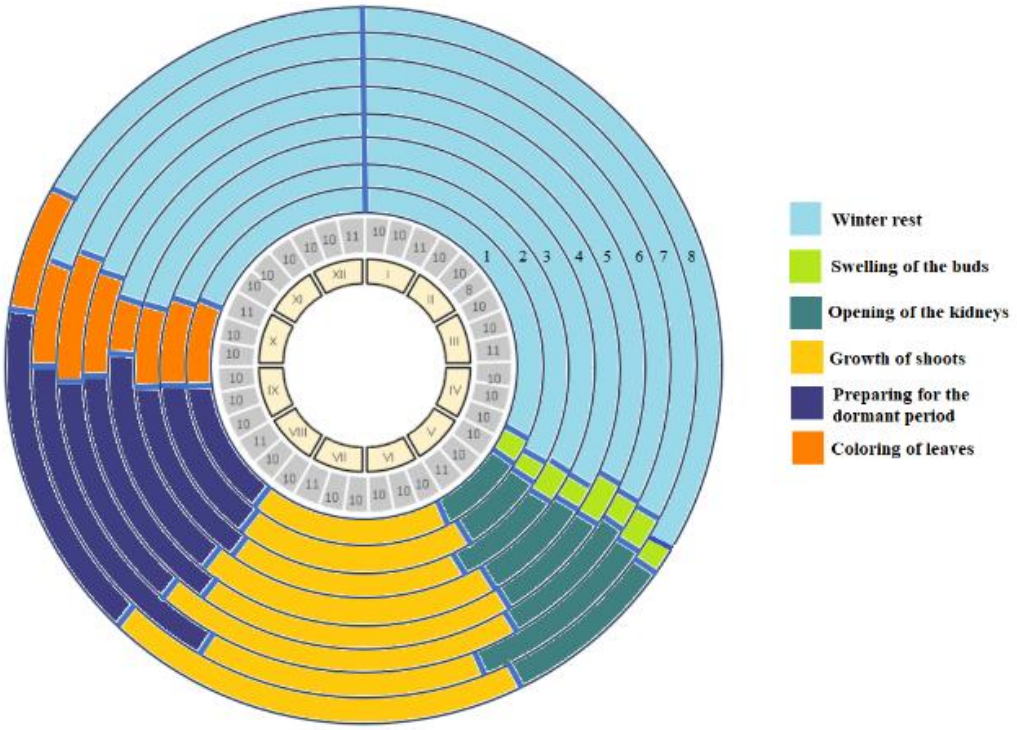

Figure 6. Diagram of the timing of the onset of phenological phases of introduced species of the genus Berberis L.: 1—Vulgares; 2—Sinenses; 3—Angulosae; 4—Dasystach; 5—Integerrimae; 6—Tschonoskyanae; 7—Heteropodae; 8—Crataeginae.

The beginning of active bud swelling is observed in the third ten-day period of April in all sections. Leaf blossoming occurs in the first ten-day period of May, 7–10 days after the bud swelling phase.

According to the start and end dates of shoot growth, introduced species are divided into three groups:

- with a late start, in the second ten days of June, and early completion of shoot growth, in the third ten days of July—*B. heteropoda* (PR);
- with a long period of shoot growth—with an early start of shoot growth, in the first ten days of June and a late end of shoot growth, in the second ten days of August—*B. crataegina* (RP);
- for other species, the period of shoot growth is observed from the first ten days of June to the first ten days of August (RS).

A late start of lignification of shoots (end of the third decade of June, first decade of July) is observed in plants from the East Asian, Central Asian and Central Asian-Kazakhstan regions.

The end dates of lignification reflect the readiness of introduced species for the winter period and affect the winter hardiness grades. Incomplete maturation of shoots (75%) is typical for the Crataeginae section. In

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other sections, the degree of lignification of annual shoots is 100%. The phase occurs in the third ten-day period of August and lasts 25–30 days.

One of the most important indicators of the degree of adaptation of introduced species to new conditions is fruiting [21, 22]. All introduced species enter the generative phase. Mass flowering occurs in the second half of June. Single flowering and fruiting is observed in *B. crataegina*, which indicates a reduced indicator of the degree of adaptation. In general, intraspecific differences in the passage of introduced species through the generative phase are preserved.

In the sharply continental climate of the introduction area, autumn leaf colouring and leaf fall indicate the transition of the introduced species to a dormant state and depends on the onset of the first autumn frosts [23]. Natural leaf fall is observed only in *B. sibirica* Pall. in the third ten-day period of September. For other species, leaf coloring is typical in the third ten-day period of September and forced leaf fall in the second half of October.

The fact that barberries go through a full development cycle indicates the success of their introduction.

The adaptation level was assessed based on eight main biological indicators: shoot lignification, winter hardiness, growth form retention, shoot formation, height gain, generative development, possible reproduction methods, and drought resistance. These criteria characterize the success of introduction and reflect the adaptive capabilities of introduced species to new environmental factors. Results of the prospects of introduced species of the genus *Berberis* L. are shown in Figure 7.

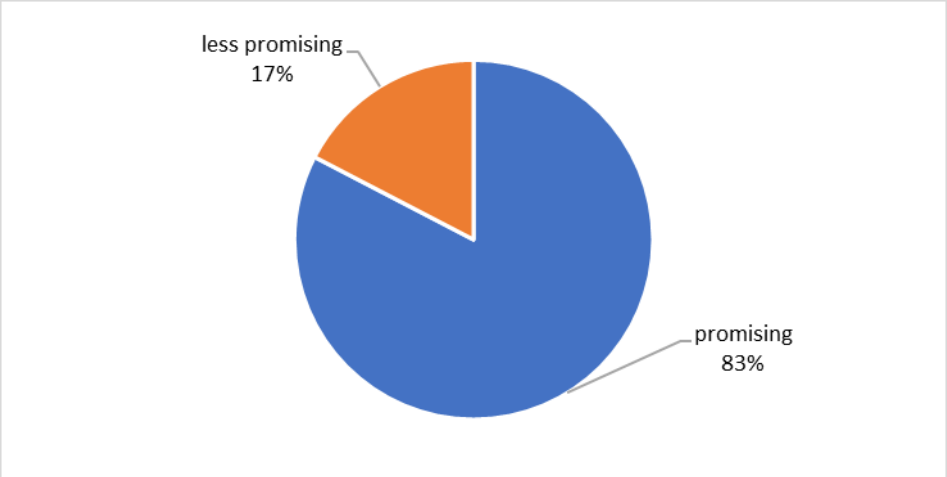

Figure 7. Evaluation of the potential of introduced species of the genus *Berberis* L.

Based on the results of the assessment of the prospects of introduced species, two groups of prospects of the genus *Berberis* L. were identified: promising and less promising. The less promising group includes *B. chinensis* Poir., *B. diaphana* Maxim., *B. vernae* Schneid., which grow in the European-Caucasian and Central Asian regions. These introduced species are characterized by reduced winter hardiness and drought resistance. The promising group includes the remaining species of the genus *Berberis* L., with high winter hardiness, preserving the genetically determined life form, with the annual passage of all phenophases, possessing high drought resistance and producing viable seeds.

Thus, the primary test of the genus *Berberis* L. in the conditions of the Altai Botanical Garden can be considered successful. The species involved showed high adaptive abilities in uncharacteristic growing conditions.

Based on this assessment, introduced species are recommended for use in phytomelioration, gardening and park construction, as ornamental and fruit crops in Eastern Kazakhstan.

Conclusions

Based on the results of verification of the collection fund of the genus *Berberis* L. of the Altai Botanical Garden, according to taxonomic ranks, 30 species, including varietal ones, belong to 8 sections, 3 subsections.

According to their affiliation with the floristic regions of their natural habitats, introduced species of the genus *Berberis* L. are classified into 6 regions: Central-South American, North American, East Asian, Central Asian, Central Asian-Kazakhstan and European-Caucasian.

According to their soil fertility requirements, the introduced species are divided into three groups: oligotrophs 17 (57%), eutrophs 7 (23%) and mesotrophs 6 (20%).

Based on moisture conditions, three groups were identified: xerophytes 9 (30%), mesophytes 8 (27%) and one intermediate group—mesoxerophytes 13 (43%).

Changes in biometric indicators under introduction conditions have been established. In the Angulosae section, an increase in bush height by 5–10% is observed, in the Integerrimae section, a decrease in bush height of up to 40% is noted, in the remaining sections, the height decreases from 20 to 35%. The crown diameter is maintained in all sections due to the good shoot-forming ability of the genus, with the exception of *B. chinensis.* from the section Sinenses. According to the annual average growth in height and trunk diameter of introduced species, annual recovery is noted in all sections. High adaptive abilities after overwintering are distinguished for the sections Vulgares, Dasystach, Tschonoskyanae, Crataeginae.

The level of adaptation of introduced species by the limiting factor—winter hardiness—is determined. Winter hardiness of the II point with partial damage to the annual growth is noted in most sections. Average winter hardiness of the III point with complete damage to the annual growth is noted in the Sinenses and Angulosae sections. In general, introduced species also show sufficient winter hardiness, which indicates high ecological plasticity of the genus.

Analysis of phenological observations shows that the passage of the entire vegetation cycle from the bud swelling phase to the end of leaf fall is extended in introduced species. The duration of the vegetation period in the arboretum of the Altai Botanical Garden is 171–191 days.

Determination of calendar dates of seasonal development of introduced species of the genus *Berberis* L., the beginning and end of the growing season, the duration of shoot growth, the degree of lignification, flowering and fruiting of all studied species have practical benefits in landscaping populated areas, use in household plots, as ornamental and fruit crops. The completion of the full development cycle by introduced species indicates the success of their introduction and resistance to unfavourable environmental conditions.

Based on the results of the assessment of the prospects of introduced species, two groups of prospects of species of the genus *Berberis* L. were identified: promising and less promising.

Thus, the initial testing of the genus *Berberis* L. in the Altai Botanical Garden can be considered successful.

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Author Contributions

The manuscript was written with the participation of all authors. All authors approved the final version of the manuscript. **Isakova E.A.** – Development of relevance, Data collection and processing, Research; **Vinokurov A.A.** – Data curation, Formal analysis, Methodology; **Danilova A.N.** – Supervision, Writing of the draft; **Lagus O.A.** – Generalization of conclusions, Editing.

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Е.А. Исакова, А.А. Винокуров, А.Н. Данилова, О.А. Лагус

Алтай ботаникалық бағы жағдайында *Berberis* L. туысына жататын жерсіндірілген ағаш өсімдіктердің бейімделуін және фенологиялық дамуының өзгергіштігін зерттеу

Мақалада Алтай ботаникалық бағы жағдайында алғашқы жерсіндіру кезінде *Berberis* L. туысының фенологиялық дамуы мен бейімделу мүмкіндіктерінің ерекшеліктері қарастырылған. *Berberis* L. туысы коллекциялық таксондарының шығу тегі сипатталып, 2025 жыл бойынша коллекциялық қордың таксономиялық құрамы нақты анықталған. Интродуценттер топырақтық-географиялық шығу тегіне қарай бес топқа бөлінген. *Berberis* L. туысына экологиялық-географиялық және морфобиологиялық сипаттамалар негізінде интродукциялық бағалау жүргізілген. Көпжылдық фенологиялық бақылаулар негізінде интродуценттердің даму циклін толық өткені анықталып, олардың бейімделу әлеуеті бағаланған. Бағалау нәтижесінде интродуценттердің болашағы бойынша екі топ айқындалды: перспективті түрлер (83 %) және аз перспективті түрлер (17 %). Осы бағалау негізінде интродуценттер Шығыс Қазақстанда фитомелиорация, бағбандық және саябақ құрылысында сәндік және жемісті дақылдар ретінде қолдануға ұсынылады.

Кілт сөздер: жерсіндіру, коллекциялық қор, *Berberis* L. туысы, болашағы, бейімделу, фенологиялық фазалар.

Е.А. Исакова, А.А. Винокуров, А.Н. Данилова, О.А. Лагус

Исследование адаптивных состояний и изменчивости фенологического развития интродуцированных древесных растений рода *Berberis* L. в условиях Алтайского ботанического сада

В статье рассматриваются особенности фенологического развития и адаптационные возможности рода *Berberis* L. при первичной интродукции в условиях Алтайского ботанического сада. Приводятся характеристики происхождения коллекционных таксонов рода, выверен таксономический состав коллекционного фонда рода *Berberis* L. по состоянию на 2025 год. Проведено деление интродуцентов по почвенно-географическому происхождению, где интродуценты распределены на 5 групп. Дана интродукционная оценка рода *Berberis* L. по эколого-географическим, морфобиологическим характеристикам. Оценен адаптационный потенциал интродуцентов на основе многолетних фенологических наблюдений, в ходе которых установлено прохождение полного цикла развития. По результатам оценки перспективности интродуцентов выявлено две группы перспективности видов: перспективные (83 %) коллекционного фонда и менее перспективные (17 %). На основе данной оценки интродуценты рекомендованы к применению для фитомелиорации, садоводства и садово-паркового строительства, как декоративные и плодовые культуры в Восточном Казахстане.

Ключевые слова: интродукция, коллекционный фонд, род *Berberis* L., перспективность, адаптация, фенологические фазы.

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Comparative analysis of the COMET, FISH, and TUNEL methods for assessing DNA damage in plants under abiotic and biotic stresses

Research that investigates plant DNA damage caused by various stress factors represents an important area of modern molecular biology and genetics. In recent decades, there has been active development of methods that allow for detailed analysis of molecular responses in plants to abiotic and biotic stresses, significantly deepening our understanding of the mechanisms underlying their adaptation to adverse conditions. One of the key aspects of such studies is the assessment of damage to genetic material, which plays an important role in disrupting the normal functioning of plant cells and tissues. Special attention is paid to the combined effects of stress factors such as high fever and viral infections, such as Tobacco bushy stunt virus (TBSV) infection, which can significantly disrupt DNA integrity and normal cellular processes. This, in turn, can lead to changes in the activity of key genes, DNA repair, as well as effects on the physiological and morphological characteristics of plants. In this article, we examined three methods that are actively used to assess DNA damage under combined stress conditions: the COMET, TUNEL and FISH. These methods allow for a comprehensive analysis of DNA damage, as well as to investigate their relationship to physiological and cellular changes in plants exposed to viral and temperature stress. The purpose of this study is to explore the prospects of using the COMET, FISH, and TUNEL assay methods to assess the level of damage to plant DNA caused by abiotic and biotic stress. The research is aimed at analyzing their effectiveness, as well as identifying advantages and limitations when working with plant objects.

Keywords: *TBSV, Nicotianabenthamiana*, combined stress, DNA damage, oxidative stress, DNA repair, COMET assay, TUNEL assay, FISH hybridization.

Introduction

Plants, as immobile organisms, are constantly exposed to various abiotic and biotic stressors, such as high temperatures, drought, UV radiation, and pathogens, including viruses. One of the most serious consequences of stress is DNA damage, which can lead to mutations, genomic instability, and even cell death [1–4]. Scenarios of combined stress (e.g., heat plus viral infection) are especially impactful because the factors act synergistically to intensify oxidative damage, perturb replication and repair, and reprogram stress-responsive gene networks [5, 6]. Given the broad adoption of *Nicotiana benthamiana* as a model for plant–microbe and plant-virus interactions, and its recently improved reference genome, the system is well-suited to dissect stress-induced genome instability [7].

One of the widely studied viruses that have a significant effect on plants is Tobacco bushy stunt virus (TBSV), a virus with positive single-stranded RNA. TBSV affects various plant species, including Nicotianabenthamiana, Arabidopsis thaliana, and other crops. The virus enters plant cells, activating the replication mechanisms of its RNA, which can disrupt the normal functioning of cells and tissues. Infection with the virus causes the destruction of cellular structures, inhibition of metabolic activity and disturbances in the process of photosynthesis. At the same time, changes in the structure and function of the plant's DNA may occur, which in turn can lead to genetic instability and deterioration of resistance to additional stresses [8]. Concurrently, viral infection (e.g., TBSV) intensifies the burden on cellular replication and defense, often elevating reactive oxygen species (ROS) and triggering programmed cell death pathways, thereby compounding genome instability under heat-virus co-stress [5, 9].

Temperature stress has a profound effect on DNA molecules, disrupting their stability and integrity. High temperatures, being an abiotic stress, can cause DNA denaturation, which is the breakdown of hydrogen bonds between a base and a complementary base in a double-stranded DNA molecule. At temperatures above 40–42 °C, the double helix collapses, leading to the formation of single-stranded fragments, which, in turn, can create "hot spots" for subsequent damage [6, 10, 11]. DNA denaturation activates cellular signaling pathways, including single-strand break repair systems and repair of damaged areas. Temperature stress can

also alter the tertiary structure of chromatin, which initiates adaptive mechanisms of cells aimed at maintaining genome stability [12].

Disruption of replication is another important aspect of exposure to temperature stress. At high temperatures, DNA denaturation occurs, preventing the normal functioning of replicative enzymes such as DNA polymerases and disrupting the replication process. This can lead to the arrest of replication and the formation of double-stranded breaks (DSBs), which require intensive repair. Double-stranded breaks are among the most dangerous DNA damages, as they can lead to serious losses of genetic information and disruption of cellular functions [13]. Excessive repair of breaks and the lack of normal replication can also disrupt the cell cycle, especially in critical areas, which causes delays in the passage of the cell cycle and can lead to the accumulation of mutations [14].

Under conditions of extreme stress, the repair mechanisms may be insufficient. The main repair systems, such as excision of damaged bases and repair of double-stranded breaks through restrictases and kinases, are activated, but their effectiveness decreases under severe temperature stress [13]. This process can lead to the accumulation of structural changes in chromosomes, which disrupts the stability of the genome and increases the likelihood of mutagenesis and cell death.

Particular attention should be paid to the combined effects of viral infection and temperature stress, as this interaction can significantly enhance molecular damage. For example, the TBSV virus (Tomato bushy stunt virus) disrupts the balance between viral RNA replication and plant genome replication. Under conditions of viral infection and temperature stress, the formation of DNA breaks increases, which is aggravated by a deficiency of repair mechanisms, leading to increased genomic instability. Viral replication requires significant energy expenditure, which can lead to an increase in oxidative stress, and together with increased temperature, this creates a critical situation for repairing DNA damage.

Analysis of molecular mechanisms

From the above analysis, it can be seen that the effects of temperature stress on plants lead to multiple molecular damages, including DNA denaturation, oxidative damage, and replication disorders. These injuries activate cellular repair mechanisms, but their effectiveness strongly depends on the degree of stress. In turn, viral infections such as TBSV enhance this effect by increasing the load on cellular DNA replication and disrupting the balance between viral and cellular RNA replication.

DNA damage includes single- and double-stranded breaks, base modifications, apurine/apyrimidine sites, and inter-stranded crosslinking [15]. Reactive oxygen species (ROS) react to the effects of viruses and high temperatures, which contribute to oxidative damage to DNA [16, 17]. For example, a significant increase in 8-oxoguanine, one of the main markers of oxidative damage, is observed during heat stress [18].

The combined effects of thermal and viral stress lead to a synergistic effect: increased ROS activity in heat conditions weakens the plant's antioxidant system, while the virus disrupts the regulation of the cell cycle and repair processes [19, 20].

This highlights the importance of research aimed at elucidating deeper molecular mechanisms of interaction between viruses and stressors, as well as developing methods to protect plants from these stresses.

Methods of DNA damage investigation

Modern methods of studying the effects of stress on plants, changes in reactive oxygen species (ROS), and DNA repair processes include several key approaches. One of the most common methods is fluorescence microscopy, which makes it possible to visualize the localization and level of ROS in plant cells using fluorescent sensors. This method allows us to track the dynamics of ROS formation in response to stress, which is important for understanding the mechanisms of cellular adaptation to adverse conditions.

Enzymatic assays also play a significant role in assessing the antioxidant activity of plants. Measuring the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase, and peroxidase allows us to study the plant's ability to detoxify ROS and its protective mechanisms. These enzymes play an important role in repairing damage caused by oxidative stress, and their activity serves as an indicator of cellular resistance to stress.

Molecular biological methods such as polymerase chain reaction (PCR) and sequencing are widely used to analyze the expression of genes related to DNA repair. Genetic studies reveal changes in the expression of key genes encoding enzymes involved in repairing DNA damage. This makes it possible not only to investigate the mechanisms of repair, but also to identify molecular markers of plant resistance to various stress factors.

In practice, three single-cell/genome-architecture methods are widely used and complementary: the COMET (single-cell gel electrophoresis) for detecting strand breaks and selected base lesions; the TUNEL for apoptosis-associated DNA fragmentation; and the FISH for chromosomal aberrations and spatial genome organization [5, 21, 22, 23].

The COMET method (or Single Cell Gel Electrophoresis, SCGE) is a highly sensitive and effective method for assessing DNA damage at the level of individual cells.

It is based on the electrophoretic migration of DNA fragments forming a characteristic "comet" [21, 24]. In the case of DNA damage, such as breaks, the DNA molecule becomes less compact, which leads to its migration and the formation of a comet-like shape, which is why this method got its name. Cells exposed to stress (for example, radiation, chemicals, temperature stress) are first suspended in an agarose gel. The gel undergoes electrophoresis, while the damaged DNA migrates towards the anode, forming a "tail" (destroyed DNA fragments). After DNA staining using fluorescent dyes, the resulting "comets" are examined under a microscope. The method is used to assess DNA damage caused by various abiotic and biotic stresses, to study mutagenic effects on cells, and to analyze the effectiveness of DNA repair. For plant systems, community guidelines emphasize pre-analytical standardization (tissue type, embedding, lysis, electrophoresis conditions) to ensure reproducibility [25], while recent reviews consolidate plant-specific applications across abiotic and biotic stresses [5, 26]. Beyond laboratory models, the COMET is being used in biomonitoring and sustainability contexts, including climate-change biology and environmental genotoxicity screening [27, 28, 29].

The COMET (Single Cell Gel Electrophoresis, SCGE) method is a highly sensitive tool for assessing DNA damage at the level of individual cells. One of the main advantages of this method is its ability to detect even minor DNA damage, such as single-stranded and double-stranded breaks, as well as base modifications. This method is used to study the effects of various stress factors, such as radiation, chemicals, and temperature stresses [30]. The advantages of the COMET method are its high sensitivity, as it is able to detect DNA damage at the level of individual cells. It is also a simple and affordable method that does not require complex equipment and can be used in laboratories with basic equipment. The COMET method is universal and can be used to assess DNA damage in both plant and animal cells. However, the method has limitations. The method is highly sensitive and applicable for the quantitative assessment of damage [31], but it does not allow distinguishing the types of damage and requires standardization of the conditions [32]. It may also be less informative for assessing the structure of the genome and the detailed localization of genetic damage.

The TUNEL method is used to identify cells in which apoptosis (programmed cell death) occurs. It allows the detection of single-stranded breaks in DNA, which are characteristic signs of apoptosis. During apoptosis, DNA fragmentation occurs in the cell, and free 3'-hydroxyl groups are formed at the ends of these fragments. This method is based on labeling the free 3'-OH ends of DNA breaks [9, 33, 34]. Fluorescent or radioactive dNTP molecules are added to label these ends using the enzyme terminal deoxynucleotidyl transferase (TdT). After that, the marks can be visualized using fluorescence microscopy or other methods. This method is used to analyze apoptosis in cells and tissues, assess DNA damage caused by external factors such as radiation or viral infections, as well as to study the mechanisms of cell death in various organisms [35]. Manipulating pro-/anti-apoptotic regulators (e.g., BAG-family genes) further illustrates how cell-death pathways intersect with stress resilience in crops [36].

The advantages of this method are its ability to accurately detect DNA fragmentation, which is characteristic of apoptosis, and to provide quantitative data on cells undergoing apoptosis [35]. The TUNEL method has a high specificity for apoptosis, which makes it possible to accurately detect DNA damage caused by cell death, and is a powerful tool for studying the mechanisms of apoptosis and progressive DNA damage in cells, which is especially useful when studying the body's response to various stresses. However, this method has limitations. It does not allow detecting DNA damage in living cells, as it requires sample fixation. In addition, it only tests for the same type of DNA damage (breaks at the ends of DNA fragments), which limits its use for analyzing other types of damage. TUNEL is sensitive to the late stages of apoptosis, but can give false positive results in the presence of necrotic lesions [37].

The FISH (fluorescent in situ hybridization) method is used to visualize specific regions of chromosomes.

The method is particularly useful for detecting chromosomal aberrations and genome instability [22, 23, 38, 39]. This method is based on the specific binding of fluorescently labeled probes to the corresponding DNA or RNA regions. DNA is fixed in cells or tissues, which is then denatured to separate the

chains. Specific single-stranded oligonucleotides labeled with fluorescent dyes are added to the sample and bind to complementary DNA regions. Measuring fluorescence using a microscope makes it possible to localize these areas and examine their distribution throughout the cell. The FISH method is used to study the structure of chromosomes, including locating genes and other sequences, as well as to evaluate chromosomal abnormalities such as deletions, duplications, and translocations. In addition, the method is used to study the distribution of specific genes or viral genomes in cells [40]. Methodological notes for cereals and other taxa highlight practical considerations for denaturing vs non-denaturing protocols and sample quality control [41].

The FISH method (fluorescent in situ hybridization) allows us to study the spatial organization of the genome, as well as analyze the localization of specific genes and viral genomes. It has high accuracy, as it allows localization of certain DNA sequences directly in cells or tissues. This method is widely used to detect chromosomal abnormalities such as deletions, duplications, and translocations [40]. The FISH method allows precise localization of specific DNA regions in chromosomes, which provides a deep understanding of the structure of the genome. It can be used in both plant and animal cells, providing information about chromosomal abnormalities such as deletions, duplications, and translocations, which is important for genetic research and diagnosis. However, the method has limitations. It requires high precision in sample preparation [42] and can be difficult to work with degraded samples. In addition, the method can be time-consuming and require highly qualified personnel to interpret the results. FISH is also limited in its use to detect DNA damage if it is not located in the area of the genes or chromosome regions of interest.

Integrated use under combined stress conditions. COMET, TUNEL, and FISH together provide a multi-angle assessment of stress impacts: COMET detects overall strand-break burden and oxidative lesions; TUNEL quantifies apoptosis-associated DNA fragmentation; FISH identifies structural chromosomal rearrangements and spatial genome alterations. In combined heat-virus scenarios in model hosts such as N. benthamiana, such an integrated panel can reveal elevated DNA migration (COMET), extensive TUNEL positivity in affected tissues, and FISH-detectable rDNA/centromere instability—linking molecular lesions to cytological outcomes [5, 7, 23].

These methods have different applications in molecular biology and genetics, and each provides unique information about DNA damage and genome structure. The COMET method is suitable for assessing DNA damage at the cellular level, TUNEL helps to study apoptosis and DNA fragmentation, and FISH helps to analyze the localization of genetic sequences and chromosomal abnormalities [43, 44]. In the context of combined stress, the use of a combination of all three methods is the most informative [45]. For example, when analyzing Nicotiana benthamiana exposed to thermal and viral stress, COMET revealed a sharp increase in DNA migration, which indicates the presence of multiple breaks [45], whereas TUNEL confirmed active apoptosis in mesophyll cells [46], and FISH showed rDNA instability and signals of loss of centromeric regions [47].

However, each method has its limitations, such as the need to prepare high-quality samples or the difficulty in interpreting the results, which requires knowledge and experience from the researcher.

Conclusion

Combined abiotic and biotic stresses, including heat and viral infection, impose multi-layered burdens on plant genomes, from oxidative base damage and strand breaks to large-scale chromosomal instability. Applying COMET, TUNEL, and FISH in concert yields complementary evidence spanning single-cell DNA damage, apoptosis-associated fragmentation, and chromosomal architecture, informing mechanism-driven strategies for improving stress resilience [5, 6, 34, 48].

The study showed that the combined effects of various stress factors, such as temperature stress and viral infections, significantly affect plants at the molecular level, which puts cells in a critical position where defense mechanisms such as heat shock proteins and DNA repair systems may not be able to cope with damage. This highlights the importance of research aimed at elucidating deeper molecular mechanisms of interaction between viruses and stressors, as well as developing methods to protect plants from these stresses.

Using the COMET, TUNEL, and FISH methods allows you to obtain comprehensive information about the types and extent of damage. The COMET method effectively evaluates DNA damage at the level of individual cells, allowing us to analyze the effects of various stressors, including radiation, chemicals, and temperatures. The TUNEL method makes it possible to assess apoptosis-related damage and analyze the mechanisms of cell death, while the FISH method allows for detailed investigation of the genome structure, identification of chromosomal abnormalities and gene localization. Each of these methods has its advantages and

disadvantages, and the choice of a specific approach depends on the objectives of the study, the type of samples and available resources.

Thus, there is a comprehensive approach to stress research, including viral infections and temperature stress, and these data are critically important for understanding plant resistance mechanisms and developing stress-resistant varieties [2, 15, 49, 50].

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Z. Turarbekova*** (Corresponding author) – Conceptualization; Methodology (overall comparative framework; COMET protocol standardization); Investigation (sample preparation and COMET runs); Visualization (figures and workflow diagram); Writing – original draft; Writing – review & editing; **N. Iksat** – Methodology (TUNEL assay setup and optimization); Investigation (TUNEL staining and microscopy); Validation (assay controls); Writing — review & editing; **A. Madirov** – Methodology (FISH probe design and hybridization workflow); Investigation (FISH imaging); Formal analysis (image quantification); Data curation; Writing — review & editing; **A. Tuyakbayeva** – Methodology (plant growth conditions and combined stress treatments — heat and viral inoculation); Investigation (stress application and sampling); Validation (biological replicates and controls); Data curation (metadata and sample tracking); Writing — review & editing; **Z. Masalimov** – Supervision; Conceptualization (study design and scope); Project administration; Funding acquisition; Resources (laboratory infrastructure and reagents); Writing — review & editing.

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Абиотикалық және биотикалық кернеулер кезінде өсімдіктердегі ДНҚ-ның зақымдануын бағалау үшін COMET, FISH және TUNEL әдістерін салыстырмалы талдау

Өсімдіктердің ДНҚ-сын әртүрлі стресс факторларының әсерінен зақымдануын зерттеуге бағытталған зерттеулер қазіргі заманғы молекулалық биология мен генетиканың маңызды бағыттарының бірі. Соңғы онжылдықтарда өсімдіктердің абиотикалық және биотикалық стрестерге молекулалық реакцияларын егжей-тегжейлі талдауға мүмкіндік беретін әдістер белсенді дамып, өсімдіктердің қолайсыз жағдайларға бейімделу механизмдерін тереңірек түсінуге жол ашты. Мұндай зерттеулердің негізгі аспектілерінің бірі — генетикалық материалдың зақымдану деңгейін бағалау, себебі бұл өсімдік жасушалары мен тіндерінің қалыпты қызметін бұзуға айтарлықтай әсер етеді. Әсіресе, жоғары температура мен вирустық инфекциялар (мысалы, Tobacco bushy stunt virus — TBSV) сияқты стресс факторларының біріктірілген әсеріне ерекше назар аударылады, себебі олар ДНҚ-ның тұтастығын бұзып, жасушалық процестердің қалыпты жүруіне кедергі келтіруі мүмкін. Бұл өз кезегінде ДНҚ-ны қалпына келтіру, негізгі гендердің белсенділігі мен өсімдіктердің физиологиялық және морфологиялық ерекшеліктеріне әсер етуі мүмкін. Мақалада біріктірілген стресс жағдайларында өсімдік ДНҚ-сындағы зақымдануды бағалауда жиі қолданылатын үш әдіс қарастырылды: COMET (сілтілі гельдік электрофорез), TUNEL (терминальді дезоксинуклеотидилтрансфераза арқылы нуклеотидтердің тізбекті жалғануы) және FISH (флуоресценттік in situ гибридизация). Бұл әдістер ДНҚ зақымдануын кешенді түрде талдауға, сондай-ақ вирустық және температуралық стресс

жағдайларына ұшыраған өсімдіктердегі физиологиялық және жасушалық өзгерістермен байланысын зерттеуге мүмкіндік береді. Бұл зерттеудің мақсаты — өсімдіктерге абиотикалық және биотикалық стресс факторларының әсері кезінде ДНҚ-ның зақымдану деңгейін бағалау үшін COMET, FISH және TUNEL талдау әдістерін қолдану мүмкіндіктерін зерттеу. Зерттеу бұл әдістердің тиімділігін талдауға, сондай-ақ өсімдік нысандарымен жұмыс істеу кезінде олардың артықшылықтары мен шектеулерін анықтауға бағытталған.

Кілт сөздер: TBSV, Nicotiana benthamiana, біріктірілген стресс, ДНҚ зақымдануы, тотығу стресі, ДНҚ репарациясы, COMET-талдау, TUNEL-талдау, FISH-гибридизация.

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Сравнительный анализ методов COMET, FISH и TUNEL для оценки повреждений ДНК растений при абиотических и биотических стрессах

Исследования, направленные на изучение повреждений ДНК у растений под воздействием различных стрессовых факторов, являются важным направлением современной молекулярной биологии и генетики. В последние десятилетия наблюдается активное развитие методов, позволяющих детально анализировать молекулярные реакции растений на абиотические и биотические стрессы, что значительно углубляет понимание механизмов их адаптации к неблагоприятным условиям. Одним из ключевых аспектов таких исследований является оценка повреждений генетического материала, поскольку они играют важную роль в нарушении нормального функционирования клеток и тканей растений. Особое внимание уделяется комбинированному воздействию стрессовых факторов, таких как высокая температура и вирусные инфекции, например заражение вирусом кустистой карликовости томата (*Tobacco bushy stunt virus*, TBSV), способных существенно нарушать целостность ДНК и нормальные клеточные процессы. Это, в свою очередь, может приводить к изменениям в активности ключевых генов, нарушению процессов репарации ДНК, а также оказывать влияние на физиологические и морфологические характеристики растений. В данной статье рассмотрены три метода, активно применяемые для оценки повреждений ДНК в условиях комбинированного стресса: COMET (щелочной гель-электрофорез), TUNEL (метод терминальной дезоксинуклеотидилтрансферазы) и FISH (флуоресцентная *in situ* гибридизация). Эти методы позволяют проводить комплексный анализ повреждений ДНК, а также исследовать их взаимосвязь с физиологическими и клеточными изменениями у растений, подвергшихся воздействию вирусного и температурного стресса. Целью данного исследования является изучение перспектив применения методов COMET, FISH и TUNEL для оценки степени повреждения ДНК растений под влиянием абиотических и биотических стрессов.

Ключевые слова: TBSV, Nicotianabenthamiana, комбинированный стресс, повреждение ДНК, окислительный стресс, репарация ДНК, COMET-анализ, TUNEL-анализ, FISH-гибридизация.

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Molecular phylogenetic analysis of six *Ribes* L. species from Kazakhstan based on DNA barcodes of nuclear and chloroplast genomes

Ribes L. species are of significant ecological and economic importance. Their berries are rich in functional metabolites, which contribute to both their nutritional value and potential health benefits. However, the genus is taxonomically complex and requires comprehensive studies to resolve its phylogenetic relationships. In this study, we sequenced three genetic regions—the internal transcribed spacer (*ITS*) and the chloroplast genes *matK* and *rbcL* to investigate the phylogeny of *Ribes* species collected in Kazakhstan. There were six species analyzed *Ribes janczewskii* Pojark., *Ribes aureum* Pursh, *Ribes graveolens* Bunge, *Ribes nigrum* L., *Ribes rubrum* L., and *Ribes saxatile* Pall., collected from various regions of Kazakhstan. Phylogenetic trees were constructed using the Maximum Likelihood method implemented in IQ-TREE. The aligned sequence lengths were 686 base pairs (bp) for *ITS*, 750 bp for *matK*, and 498 bp for *rbcL*. Among these, the *ITS* region showed the highest number of polymorphic sites (219), followed by *matK* (195) and *rbcL* (50). Nucleotide diversity (Pi) was also the highest in the *ITS* region (0.0735), nearly double that of *matK* (0.03703), and substantially greater than *rbcL* (0.01378). The nucleotide sequences of *ITS*, *matK*, and *rbcL* obtained from this study have been deposited in the GenBank database of the National Center for Biotechnology Information (NCBI) under accession numbers PV702933-PV702944, PV730383-PV730394, and PV730395-PV730406, respectively. The newly generated sequence data provide a valuable foundation for future phylogenetic and evolutionary research in *Ribes*.

Keywords: Ribes, Kazakhstan, phylogeny, *ITS*, *rbcL*, *matK*, DNA-barcoding.

Introduction

Ribes L., a genus in the family Grossulariaceae DC., which is primarily distributed across Eurasia, North America, and parts of South America [1]. The genus comprises approximately 200 species of deciduous, perennial shrubs [2]. Commonly referred to as currants and gooseberries, members of *Ribes* are of substantial ecological, economic, and horticultural significance [3]. *Ribes* species are particularly valued for their fruits, which are consumed both fresh and in a variety of processed forms, including jams, wines, juices, and candies. This wide range of uses is attributed to the fruits' high content of functional metabolites, which contribute to their nutritional and commercial appeal [4]. Among the species, *Ribes nigrum* L. (black currant) has been extensively studied for its phytochemical composition [5–7]. These studies have demonstrated that *R. nigrum* berries are particularly rich in phenolic compounds and exhibit significant antioxidant and antimicrobial activities.

In Kazakhstan, the genus *Ribes* is represented by 11 wild species [8], including *Ribes janczewskii* Pojark., which is listed in the Red Book of Kazakhstan [9]. Among these, *Ribes aureum* Pursh, *Ribes graveolens* Bunge, *Ribes nigrum* L., *Ribes rubrum* L., and *Ribes saxatile* Pall. are the most widely distributed. Most of these species are of particular importance due to their high vitamin content and potential use in nutritional and pharmaceutical applications. Several species of *Ribes* in Kazakhstan have been the subject of research involving cryopreservation techniques [10] and molecular genetic studies [11]. In addition, numerous investigations have focused on the biochemical composition and breeding potential of various cultivated *Ribes* varieties, underscoring their value for both conservation and agricultural development [12–14].

Molecular phylogenetic analysis plays a crucial role in resolving complex evolutionary relationships, particularly in plant genera characterized by high levels of morphological convergence. *Ribes* is one such genus with a long history of taxonomic ambiguity and ongoing debate regarding its classification [2]. Despite numerous morphological and molecular studies, the taxonomy of *Ribes* remains controversial. To ad-

dress these challenges, molecular markers from both nuclear and chloroplast genomes have been widely employed in phylogenetic investigations of *Ribes* [15–17]. However, despite these efforts, the molecular taxonomy of the genus is still incomplete and requires further comprehensive studies involving broader sampling.

In this study, we sequenced the internal transcribed spacer (*ITS*) region along with the chloroplast genes *matK* and *rbcL* to evaluate the phylogenetic positions of six *Ribes* species—*Ribes janczewskii*Pojark., *Ribes aureum* Pursh, *Ribes graveolens* Bunge, *Ribes nigrum* L., *Ribes rubrum* L., and *Ribes saxatile* Pall., collected from various regions of Kazakhstan.

Experimental

Collection of the plant leaves and DNA isolation

Plant samples were collected from the southeastern, western, and eastern regions of Kazakhstan by Ivaschenko A., Imanbayeva A., and Sumbembayev A., respectively. Detailed information on the collection sites is provided in Table 1. The collected plant materials were dried using silica gel and subsequently used for DNA extraction. Genomic DNA was isolated following the cetyltrimethylammonium bromide (CTAB) protocol [18]. DNA concentration was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), and DNA quality was assessed by electrophoresis on a 1.0 % agarose gel.

Table 1

Collected sites of the studied seven *Ribes* species from Kazakhstan

Species	Collected site	Collected by
Ribes nigrum L.	Western Altai, Lineisky ridge	Sumbembayev A.
Ribes rubrum L.	Western Altai, Kholzun ridge	Sumbembayev A.
Ribes saxatile Pall.	East Kazakhstan region, Kokpekty district, Eastern Kalba ridge	Sumbembayev A.
Ribes graveolens Bunge	East Kazakhstan region, Ridder district, Koksu ridge	Sumbembayev A.
Ribes janczewskii Pojark.	Almaty region, upper reaches of Turgen	Ivaschenko A.
Ribes aureum Pursh	Mangistau region, Mangyshlak, Western Karatau, Kogez gorge	Imanbayeva A.

PCR amplification and sequencing

DNA barcoding markers, including *ITS*, *matK*, and *rbcL*, were employed for phylogenetic analysis. PCR amplification was carried out in a 20 μL reaction mixture comprising genomic DNA as the template, buffer solution, $MgCl_2$, dNTPs, forward and reverse primers, and Taq DNA polymerase. The amplifications were performed using a SimpliAmp Thermal Cycler (Thermo Fisher Scientific, USA). PCR conditions and the nucleotide sequences of the primers used were applied as described in White et al. (1990) [19] for *ITS*, Kress & Erickson (2007) [20] for *matK* and *rbcL*. Following amplification, PCR products were separated by electrophoresis on a 1.5 % agarose gel. Target bands were excised from the gel and purified using the ULTRAPrep® Agarose Gel Extraction Mini-Prep Kit (AHN Biotechnologie GmbH, Nordhausen, Germany), following the manufacturer's protocol. The purified PCR products were then sequenced in forward and reverse directions using BigDye™ Terminator Cycle Sequencing chemistry (Applied Biosystems, USA). Sequencing was carried out on an ABI 3130 Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific, USA). Two samples from each species were sequenced and included in the analysis.

Phylogenetic analysis

For the phylogenetic analysis, nucleotide sequences of *ITS*, *matK*, and *rbcL* markers were utilized. Analyses were conducted using both individual and concatenated sequence datasets. A total of 25 samples were included, comprising 12*Ribes* samples (representing six species) collected from Kazakhstan, 11*Ribes* sequences retrieved from NCBI GenBank, and two additional outgroup sequences (*Rosa laevigata* and *Rhamnus grubovii*). Sequence alignment was performed using MEGA X [21]. Phylogenetic trees were reconstructed using the Maximum Likelihood (ML) approach. The optimal nucleotide substitution models were selected based on the Bayesian Information Criterion (BIC), with the following models: TIM+F+I+R2for ITS, TPM3u+F for *matK*, and TPM2 for *rbcL* nucleotide sequences. ML analysis was conducted using IQ-TREE v2.2.2.6 [22]. The subgenus and section names were given according to Schultheis & Donoghue (2004) [16]. Schultheis & Donoghue (2004) proposed a molecular phylogenetic classification system for the genus *Ribes*, based on data from nuclear and chloroplast DNA markers [16].

Results and Discussion

In the present study, three datasets were employed for phylogenetic analysis: (1) nucleotide sequences of the internal transcribed spacer (*ITS*) region, (2) nucleotide sequences of the *matK*gene, and (3) nucleotide sequences of the *rbcL* gene. The nucleotide sequences of *ITS*, *matK*, and *rbcL* obtained in this study have been deposited in the GenBank database of the National Center for Biotechnology Information (NCBI) under accession numbers PV702933-PV702944, PV730383-PV730394, and PV730395-PV730406, respectively. Phylogenetic trees for each dataset were constructed using the Maximum Likelihood (ML) method. A total of 23 *Ribes* samples were included in the analysis, comprising 12 samples representing 6 species collected in this study, along with sequences obtained from the NCBI GenBank. Additionally, two species—*Rosa laevigata* and *Rhamnus grubovii* were used as outgroups to root the phylogenetic trees. Detailed information on all samples is provided in Table 2.

Table 2

The list of samples used in the study

Species	NCBI accession numbers		
	ITS	***matK***	***rbcL***
Ribesaciculare	AY138050.1	PQ348655.1	PQ337383.1
Ribesamericanum	AF426375.1	MK520528.1	HQ590238.1
Ribesaureum	PQ443797.1	OQ847549.1	PQ337465.1
Ribescynosbati	AY138051.1	MK520529.1	HQ590239.1
Ribesglaciale	MH710923.1	MW382543.1	MW382720.1
Ribesgraveolens	MZ366410.1	MZ361533.1	MZ361434.1
Ribeshimalense	MH711381.1	MH659873.1	JF944130.1
Ribeslacustre	AF426366.1	KX677769.1	HQ590240.1
Ribesnigrum	AF426374.1	HE967476.1	PQ337447.1
Ribesstenocarpum	AY138056.1	MW382557.1	MW382733.1
Ribesjanczewskii	PP464115.1	PP708896.1	PP493207.1
Ribesnigrum KZ 1	PV702933.1	PV730383.1	PV730395.1
Ribesnigrum KZ 2	PV702934.1	PV730384.1	PV730396.1
Ribesrubrum KZ 1	PV702935.1	PV730385.1	PV730397.1
Ribesrubrum KZ 2	PV702936.1	PV730386.1	PV730398.1
Ribessaxatile KZ 1	PV702937.1	PV730387.1	PV730399.1
Ribessaxatile KZ 2	PV702938.1	PV730388.1	PV730400.1
Ribesgraveolens KZ 1	PV702939.1	PV730389.1	PV730401.1
Ribesgraveolens KZ 2	PV702940.1	PV730390.1	PV730402.1
Ribesjanczewskii KZ 1	PV702941.1	PV730391.1	PV730403.1
Ribesjanczewskii KZ 2	PV702942.1	PV730392.1	PV730404.1
Ribesaureum KZ 1	PV702943.1	PV730393.1	PV730405.1
Ribesaureum KZ 2	PV702944.1	PV730394.1	PV730406.1
Rosa laevigata	FJ416663.2	MH552372.1	GU363797.1
Rhamnusgrubovii	KR083248.1	MZ361530.1	MZ361431.1

The Maximum Likelihood (ML) phylogenetic tree constructed from ITS nucleotide sequences divided the *Ribes* species into three primary clades, corresponding to the two major subgenera: *Ribes* and *Grossularia*. The samples of *Ribes aureum* analyzed in this study clustered together with *R. aureum* reference sequences from the NCBI GenBank, confirming their species identity. Similarly, the samples of *Ribes graveolens*, *Ribes nigrum*, and *Ribes janczewskii* formed a distinct subclade along with corresponding sequences of these species from GenBank, indicating strong phylogenetic coherence. *Ribes saxatile* samples grouped with *Ribes glaciale* from GenBank, suggesting a close genetic relationship between these taxa, indi-

cating that the *Ribes saxatile* might belong to the section *Berisia*. Additionally, *Ribes rubrum* samples from this study clustered with *Ribes himalense* sequences, indicating shared ancestry, which is consistent with their placement in the same subgenus (subg. *Ribes*) and section (sect. *Ribes*) according to the sectional classification proposed by Schultheis & Donoghue (2004). Species from the subgenus *Grossularia*—namely *Ribes aciculare*, *Ribes cynosbati*, and *Ribes stenocarpum*formed a well-supported clade together with *Ribes lacustre* (classified under the subgenus *Ribes*) from GenBank. This grouping suggests a complex evolutionary relationship between members of the two subgenera (Fig. 1). The section and subgenus names of the analyzed species were assigned according to Schultheis and Donoghue (2004) [16].

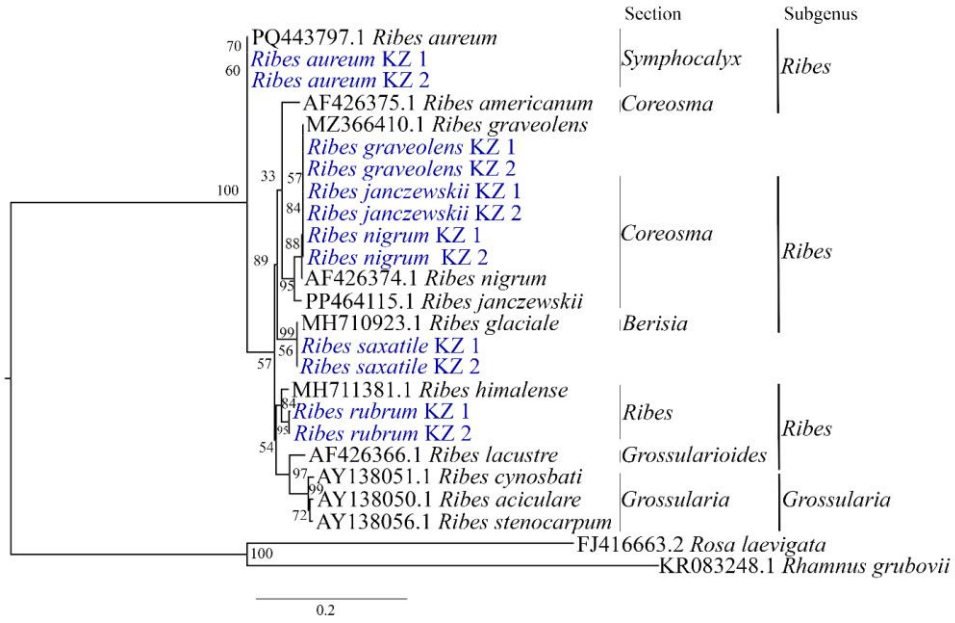

Figure 1. Maximum Likelihood phylogenetic tree based on *ITS* sequences reconstructed using 23 ingroup and 2 outgroup samples. The numbers at the branch nodes represent ML bootstrap value. The species analyzed in this study are highlighted in blue.

The ML phylogenetic trees reconstructed from the nucleotide sequences of the *matK* (Fig. 2) and *rbcL* (Fig. 3) genes exhibited similar topologies, supporting congruent evolutionary relationships among the *Ribes* species. Samples of *Ribes aureum* and *Ribes rubrum* obtained in this study clustered together with *Ribes americanum*, *Ribes himalense*, and *Ribes aureum* reference sequences from GenBank, indicating close genetic relationships among these taxa. Likewise, samples of *Ribes graveolens*, *Ribes nigrum*, and *Ribes janczewskii* from this study formed a clade with corresponding GenBank sequences of the same species. Additionally, the samples of *Ribes saxatile* from this study grouped with *Ribes glaciale* and *Ribes aciculare* from GenBank, suggesting a potential phylogenetic affinity among these species.

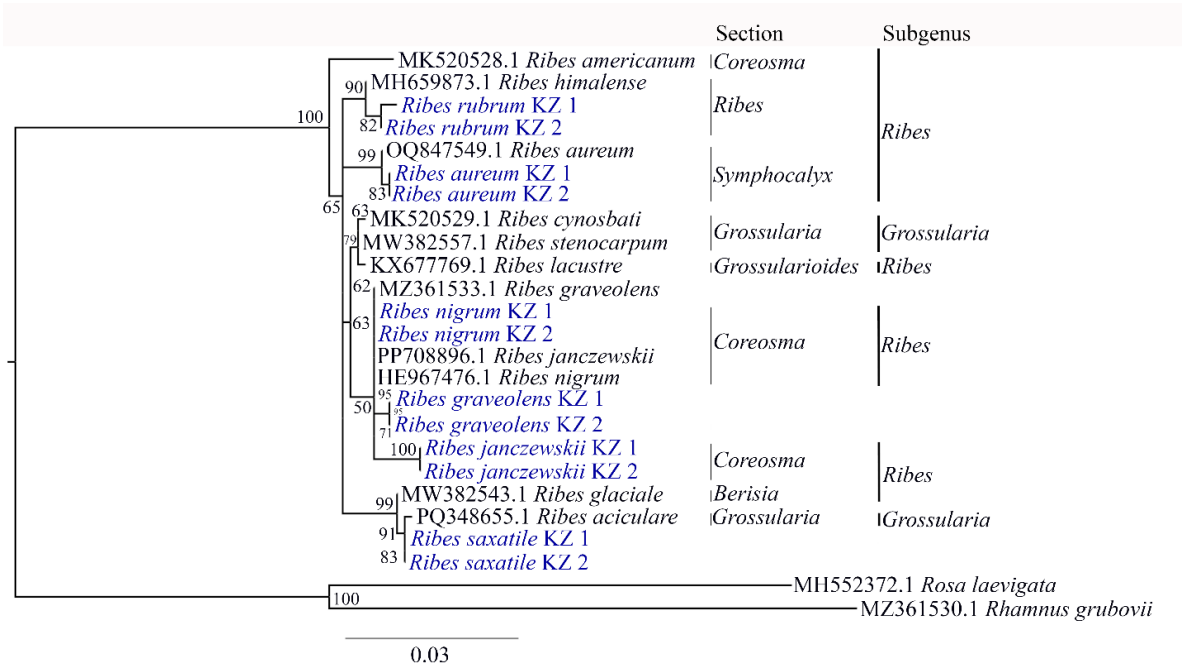

Figure 2. Maximum Likelihood phylogenetic tree based on *matK* sequences reconstructed using 23 ingroup and 2 outgroup samples. The numbers at the branch nodes represent ML bootstrap value. The species analyzed in this study are highlighted in blue.

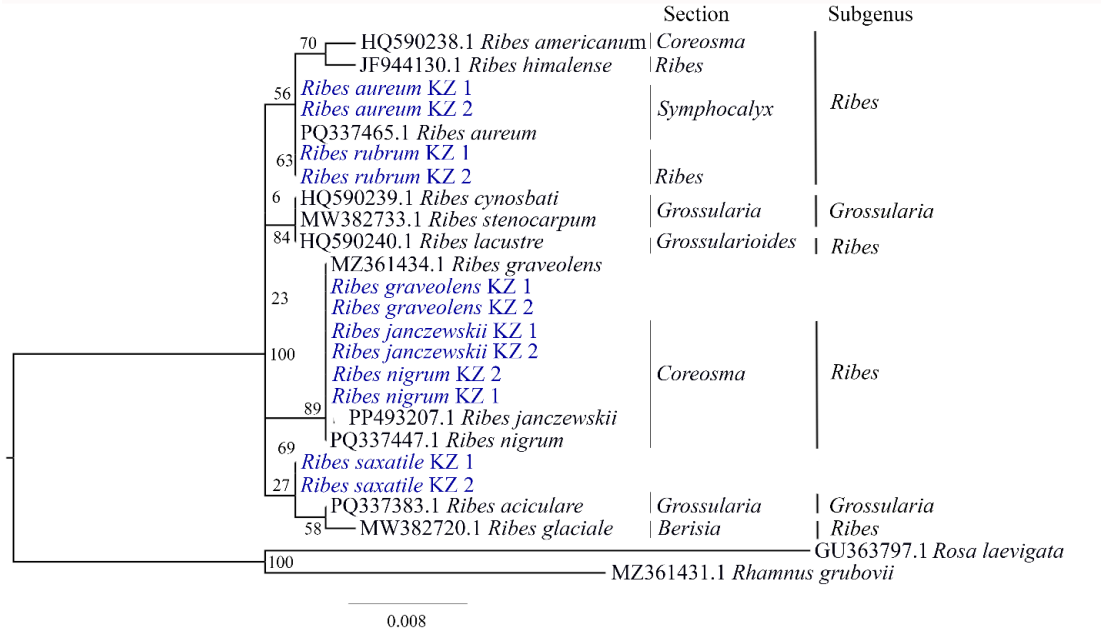

Figure 3. Maximum Likelihood phylogenetic tree based on *rbcL* sequences reconstructed using 23 ingroup and 2 outgroup samples. The numbers at the branch nodes represent ML bootstrap value. The species analyzed in this study are highlighted in blue.

Summary statistics for the aligned sequences of the *ITS*, *matK*, and *rbcL* are presented in Table 3. The aligned sequence lengths were 686 base pairs (bp) for *ITS*, 750 bp for *matK*, and 498 bp for *rbcL*. Among these, the *ITS* region exhibited the highest number of variable (polymorphic) sites (219), followed by *matK* (195) and *rbcL* (50), indicating that *ITS* was the most informative marker for detecting sequence variation in the studied *Ribes* species. A comparable number of polymorphic sites in the *ITS* region has also been reported in *Asclepias* species [23]. The number of observed haplotypes was highest for *matK* (17), slightly exceed-

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ing *ITS* (16), while *rbcL* displayed a lower haplotype count (10). Haplotype (gene) diversity (Hd) was high across all markers, with *matK* showing the greatest diversity (0.953), followed by *ITS* (0.917) and *rbcL* (0.833).

Nucleotide diversity (Pi), a measure of average pairwise sequence divergence, was highest in the *ITS* region (0.0735), nearly double that of *matK* (0.03703), and significantly higher than *rbcL* (0.01378). Similarly high Pi values exceeding 0.07 have been reported for chloroplast DNA markers in certain orchid species, such as *Caularthronbicornutum* and *Myrmecophilathomsoniana* [24], suggesting that elevated nucleotide diversity is not restricted to nuclear regions in all taxa. These results confirm that the *ITS* region provided the relatively greatest resolution for assessing variability within *Ribes*, whereas *rbcL* was the most conserved marker among the three.

Table 3

Summary of sequence characteristics and genetic diversity parameters for *ITS*, *matK*, and *rbcL* in analyzed *Ribes* species

	ITS	***matK***	***rbcL***
Aligned length	686	750	498
Variable (polymorphic) sites	219	195	50
Number of Haplotypes, h	16	17	10
Haplotype (gene) diversity, Hd	0,917	0,953	0,833
Nucleotide diversity, Pi	0,0735	0,03703	0,01378

The nucleotide sequences of the *ITS* are widely utilized in resolving phylogenetic relationships across diverse plant taxa [25–27]. The high informativeness of *ITS* has also been demonstrated in several previous studies. For example, its effectiveness in species-level discrimination has been reported in families such as Cactaceae [28], Orchidaceae [29], as well as Fabaceae and Poaceae [30]. The phylogenetic analyses conducted in this study demonstrated that the *ITS* nucleotide sequences exhibited the highest level of polymorphism among the markers tested, indicating that *ITS* is potentially more informative for resolving phylogenetic relationships among *Ribes* species than the chloroplast markers *matK* and *rbcL*. The newly generated nucleotide sequence data represent a valuable resource for future phylogenetic and evolutionary studies in *Ribes*. These findings might contribute to a better understanding of species relationships within the genus and provide a foundation for future taxonomic revisions.

Conclusions

In this study, nucleotide sequences of the nuclear internal transcribed spacer (*ITS*) and the chloroplast genes *matK* and *rbcL* were generated and utilized for phylogenetic analysis of *Ribes* species. The results indicate that the *ITS* region is the most informative among the three markers, offering the highest resolution for detecting genetic variability within the genus. In contrast, *rbcL* was found to be the most conserved marker, providing limited phylogenetic differentiation. These results underscore the effectiveness of the *ITS* region in resolving intrageneric relationships in *Ribes*. The newly obtained sequences enrich the molecular dataset for the genus and provide a foundation for future phylogenetic and evolutionary studies. However, further studies, including a broader sampling of *Ribes* species, are necessary to achieve a more comprehensive and robust understanding of the genus taxonomy.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Almerekova S.S.** — Conceptualization, Software, Validation, Formal analysis, Investigation, Data curation, Visualization, Writing — original draft, Writing — review and editing, Supervision; **Yermagambetova M.M.** — Methodology, Validation, Formal analysis, Investigation, Writing — original draft; **Sumbembayev A.A.** — Investigation, Resources; **Ivashchenko A.A.** — Investiga-

tion, resources; **Imanbayeva A.A.** — Investigation, Resources, Funding acquisition; **Turuspekov Y.K.** — Conceptualization, Data curation, Writing — review and editing, project administration.

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Қазақстандық алты *Ribes* L. түрлерінің ядролық және хлоропласт геномдарының ДНҚ-баркодтары негізінде молекулалық-филогенетикалық талдауы

Ribes L. туысы түрлері айтарлықтай экологиялық және экономикалық маңыздылыққа ие. Олардың жидектері функционалды метаболиттерге бай, бұл олардың тағамдық құндылығына да, денсаулыққа пайдалы қасиеттеріне де ықпал етеді. Алайда, бұл туыс оның филогенетикалық байланыстарын нақтылау үшін кешенді зерттеулерді қажет ететін таксономиялық күрделі топ. Зерттеуде Қазақстан аумағында жиналған *Ribes* түрлерінің филогенетикасын зерттеу мақсатында үш генетикалық аймақ — ішкі транскрипцияланатын спейсер (*ITS*) және *matK*мен*rbcL* хлоропласт гендері секвенирленді. Қазақстанның әр аймақтарында жиналған алты түр талданды: *Ribes janczewskii* Pojark., *Ribes aureum* Pursh, *Ribes graveolens* Bunge, *Ribes nigrum* L., *Ribes rubrum* L. және*Ribes saxatile* Pall. Филогенетикалық дендрограммалар IQ-TREE бағдарламасында Maximum Likelihood әдісімен жүзеге асырылды. Түзетілген тізбектердің ұзындығы *ITS* үшін 686 нуклеотидтік жұбы (н.ж.), *matK* үшін 750 н.ж. және *rbcL* үшін 498 н.ж. болды. Олардың ішінде полиморфты аймақтардың ең көп саны *ITS* аймағында (219), одан кейін *matK* (195) және *rbcL* (50) нуклеотидтік тізбектерінде анықталды. Нуклеотидтік алуантүрлілік (Pi) те*ITS* нуклеотидтік тізбегінде ең жоғары (0,0735) болды, *matK* (0,03703) мен *rbcL* (0,01378) нуклеотидтік тізбектерінен айтарлықтай жоғары екендігі айқындалды. Алынған *ITS*, *matK*, және*rbcL* нуклеотидтік тізбектері сәйкесінше PV702933-PV702944, PV730383-PV730394 және PV730395-PV730406 тіркеу нөмірлерімен Ұлттық биотехнологиялық ақпарат орталығының (NCBI) GenBank деректер базасына жүктелді. Жаңа нуклеотидтік тізбектер *Ribes* туысының болашақ филогенетикалық және эволюциялық зерттеулері үшін құнды негіз болып табылады.

Кілт сөздер: Ribes, Қазақстан, филогения, *ITS*, *rbcL*, *matK*, ДНҚ-баркодтау.

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Молекулярно-филогенетический анализ шести видов *Ribes* L. из Казахстана на основе ДНК-баркодов ядерного и хлоропластного геномов

Виды рода *Ribes* L. обладают значительной экологической и экономической важностью. Их ягоды богаты функциональными метаболитами, которые способствуют как их пищевой ценности, так и потенциальным полезным свойствам для здоровья. Однако данный род представляет собой таксономически сложную группу, требующую комплексных исследований для уточнения его филогенетических взаимосвязей. В настоящем исследовании были просеквенированы три генетических региона — внутренний транскрибируемый спейсер (*ITS*) и хлоропластные гены *matK* и *rbcL* — с целью изучения

филогенетики видов *Ribes*, собранных на территории Казахстана. Было проанализировано шесть видов: *Ribes janczewskii* Pojark., *Ribes aureum* Pursh, *Ribes graveolens* Bunge, *Ribes nigrum* L., *Ribes rubrum* L. и *Ribes saxatile* Pall., собранных в различных регионах Казахстана. Филогенетические деревья были построены методом максимального правдоподобия (Maximum Likelihood) в программе IQ-TREE. Длина выровненных последовательностей составила 686 пар нуклеотидов (п.н.) для *ITS*, 750 п.н. для *matK* и 498 п.н. для *rbcL*. Среди них наибольшее число полиморфных участков было выявлено в регионе *ITS* (219), за ним следовали *matK* (195) и *rbcL* (50). Нуклеотидное разнообразие (Pi) также оказалось наивысшим в *ITS* (0,0735), почти в два раза превышая значение для *matK* (0,03703) и значительно превосходя *rbcL* (0,01378). Полученные нуклеотидные последовательности *ITS*, *matK*, и *rbcL* были депонированы в базу данных GenBank Национального центра биотехнологической информации (NCBI) под регистрационными номерами PV702933-PV702944, PV730383-PV730394 и PV730395-PV730406 соответственно. Новые нуклеотидные последовательности представляют ценную основу для будущих филогенетических и эволюционных исследований рода *Ribes*.

Ключевые слова: Ribes, Казахстан, филогения, *ITS*, *rbcL*, *matK*, ДНК-баркодирование.

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Study of correlations between selection traits of oatgenotypes in Akmola region

The aim of the present study was to investigate the correlational relationships between valuable agronomic traits of common oat (*Avena sativa*), important for breeding programs aimed at improving the productivity of agricultural crops. The research was conducted in 2023-2024 on field experimental plots at the A. I. Barayev Scientific and Production Center for Grain Farming. Observations were carried out using biometric measurements of plant height and morphological traits related to productivity. The biological material consisted of 16 oat genotypes. The results were analyzed using correlation coefficients, analysis of variance, multiple regression, and the study of relationships between traits. It was established that productive tillering has a linear relationship with a number of traits, except for the mass and number of grains in the panicle (r = –0.39…0.46). According to the correlation analysis, the following relationships were identified: 1000-grain weight and plant height; seed mass per plant and panicle length (r = 0.38…0.46); productive tillering and seed mass per panicle (r = 0.46). These traits can be effectively used in the breeding process to develop high-yielding oat varieties. According to the results of structural analysis of productive elements, the genotypes featuring a complex of valuable traits were selected: Yarovoy and Fax varieties, as well as the FL 0524 sample. These valuable genotypes can be recommended as sources for increasing productive properties in oat plants.

Keywords: oats, correlation, selection, variety, valuable agronomic traits, vegetation period, productivity.

Introduction

Common oat (*Avena sativa*) is an annual herbaceous plant, a species of the Oats genus (*Avena*), a valuable cereal widely used in agriculture. Oat is tolerant to various soil and climate conditions, has a relatively short vegetation period (75–120 days), and its seeds germinate at +2 °C. Oat seedlings survive slight frosts up to 4-5 °C: cold tolerance of oat culture allows it to be cultivated successfully in the North [1]. Oat straw is soft and more suitable for livestock than the straw from other cereal crops. The health benefits of oats have been recognised only recently. All these characteristics offer opportunities to improve oat as a green fodder crop for intensively cultivated grazing animals and as a dual-purpose crop for resource-limited farmers [2]. According to the Bureau of National Statistics of the Republic of Kazakhstan, oats are cultivated on an area about 231.3 hectaresin our country (https://stat.gov.kz/api/iblock/element/73401/file/ru/).

The present study was conducted to evaluate oat cultivars for various forage and grain yield traits: plant height (cm), panicle length (cm), number of grains per plant, number of grains per panicle, weight of 1000 grains (g) and duration of vegetation period (days). However, to obtain a clear picture of the inheritance of various grain yield traits, the present experiment was conducted to assess the variability of common oat and to identify phenotypic correlations between grain yield and its components, as well as between individual elements.

Even if it is sown in small areas, oat is an important crop not only for its grain purposes but also for the whole food industry. Studies of extensive collections of oats show that oats have a wide genotypic and phenotypic diversity. Most productivity components, including several grains, are highly heritable. Regarding phenotypic expression, the general performance of a genotype depends on its area of origin. The genotypes in a particular region have been found to share a common genetic origin. Even if genetic diversity is limited in plant breeding, the traits that contribute to the plant's productivity maintain high genetic diversity. This also includes plant height and duration of the vegetation period. For valuable genetic resources identification, the studies which include molecular markers are instrumental: they follow and support the studies of phenotypic variability [3, 4]. The evaluation of collections to find sources for plant selection work is continuous. The effect of some traits on panicle productivity, determined by the number of grains per panicle, is significant. This trait also depends on other productivity traits and is affected by environmental conditions. In this

regard, our research aimed to study the relationship between the valuable agronomic traits in oat genotypes and to determine the traits that heavily impact plant productivity.

Experimental

The study was conducted in 2023-2024. The research materials were the following oat varieties approved for use in the different regions of Kazakhstan: Mirt, Debut, Lidya, Fax, Ural 2, SIG, Yazdyk, Desant, Antey, Baizat, Syrgalum, as well as samples FL06014, FL0524, FL0538, and FL06006 (Fig. 1).

Figure 1. Regions of the Republic of Kazakhstan where the studied oat varieties are approved for use

Methodology of field experiments. The Duman variety was used as a standard variety in every 10 samples, with three repetitions. Phenological observations, assessment of resistance to environmental stress factors, yield estimation, laboratory analysis of the plants and other indicators are carried out by the methodological VIR (Vavilov All-Russian Institute of Plant Genetic Resources) guidelines for enrichment, conservation and study of the world collection. The accuracy of assessment and estimation at all stages of the sample collection study depended on precise work performance and a correct understanding of plant development phases and their characteristics. During the phenological observations, visits to the experimental plot were repeated every 1-2 days. The observations were recorded in the field journals. General phenological observation of all the plants was carried out to ensure data comparability before plant monitoring, and field germination of plants was also recorded. Oat vegetation and development can be divided into the following phases: seed germination, emergence of seedlings, tillering, stem elongation, flowering and maturation. Related to plant adaptation and productivity component traits were analyzed: vegetation period, plant height, productive tillering, grain weight per plant, grain weight per panicle, panicle length, number of grains in panicle, weight of 1000 grains [5].

Statistical processing of the obtained data. The principal component analysis (PCA) method visualised the relationships and described the distances between the values. Conventional cluster analysis was used to group the varieties featuring the valuable agronomic traits. Coefficients were calculated using the RStudio correlation matrix to determine the relationships between the values.

The meteorological data for the growing experimental period 2023-2024 was gathered at the local weather station (Table 1).

Table 1

Location, environment, and weather data during agronomic seasons

Site/Region	Akmola Region
Latitude/Longitude	51.41′/70.59′
Soil type	dark chestnut (3.6-4.1 % humus)
Conditions	Rainfed

Continuation of Table 1

Year	2023	2024
Annual rainfall, mm	72.2	309
Mean temperature, ºC	18.1	18
Max temperature, ºC	24.4	27
Min temperature, ºC	11.8	14

Results and Discussion

The studies on identifying correlations between the elements that contribute to productivity highlight that understanding the relationships between traits benefits plant selection programmes [6]. Component traits of plant productivity have high variability, most of which are strongly impacted by climatic conditions. Even if variability is high between and within populations, there is a positive linear correlation between them; it primarily concerns such vital traits as panicle length, panicle productivity and weight of 1000 grains [7]. Correlations between traits are essential for all the types of oat plants, whether they are cultivated for fodder or grain. Grain weight per panicle, last leaf weight, protein percentage in grain and chlorophyll content in leaves were studied in addition to the number of grains per panicle. All these traits are positively correlated with grain production [8]. Genotypic correlations must complement phenotypic correlation coefficients. Plant development ensures grain production. Productive tillering, plant height and vegetative mass are the traits that contribute to grain formation in a panicle and the development of a certain amount of vegetative mass in fodder oat. Green forage mass development positively correlates with the number of leaves per plant, plant height, stem to leaf ratio, number of shoots and straw diameter. Due to this fact, all these traits can be improved simultaneously. Grain quality, which is determined by protein and fibre content, is negatively correlated with productivity elements. Grain yield is positively related to the number of seeds per panicle, the weight of 1000 grains and the grain length to width ratio. So, the selection strategy should be considered a combination of activities considering the relationships between all the traits [9]. Other experiments have underlined the importance of the interval before flowering in vegetative plant development and the ratio between foliage and stems because these traits influence yield and quality [10].

Northern Kazakhstan's climate is insufficient to ensure the reliable ripening of medium-ripening and late-ripening oat varieties, complicating the attempts to introduce oats into the region. Oat sprouts tolerate short-term temperature drops to +5 °C. Autumn frosts below +2 °C disrupt seed development and cause leaf freezing. Considering an average daily temperature below 9 °C in May and September in the Akmola region, and frosts can occur in early September, the main requirement for local oat cultivation is a short vegetation-period. In this context, one of the essential objectives of the study was to carry out selection and genetic evaluation of the gene pool and to identify promising forms for developing the new varieties suitable for cultivation in the country's northern regions.

In 2023-2024, the valuable agronomic traits of oat genotypes were evaluated comprehensively. As a result of the research, the promising varieties were selected; the selection was based on the duration of the vegetation period, grain yield per square metre, and weight of 1000 grains. There value was $p<0,05$, which means varieties did not affect the yield, but the traits had p value above 0.05, which proves the influence of varieties on the yield (Fig. 2).

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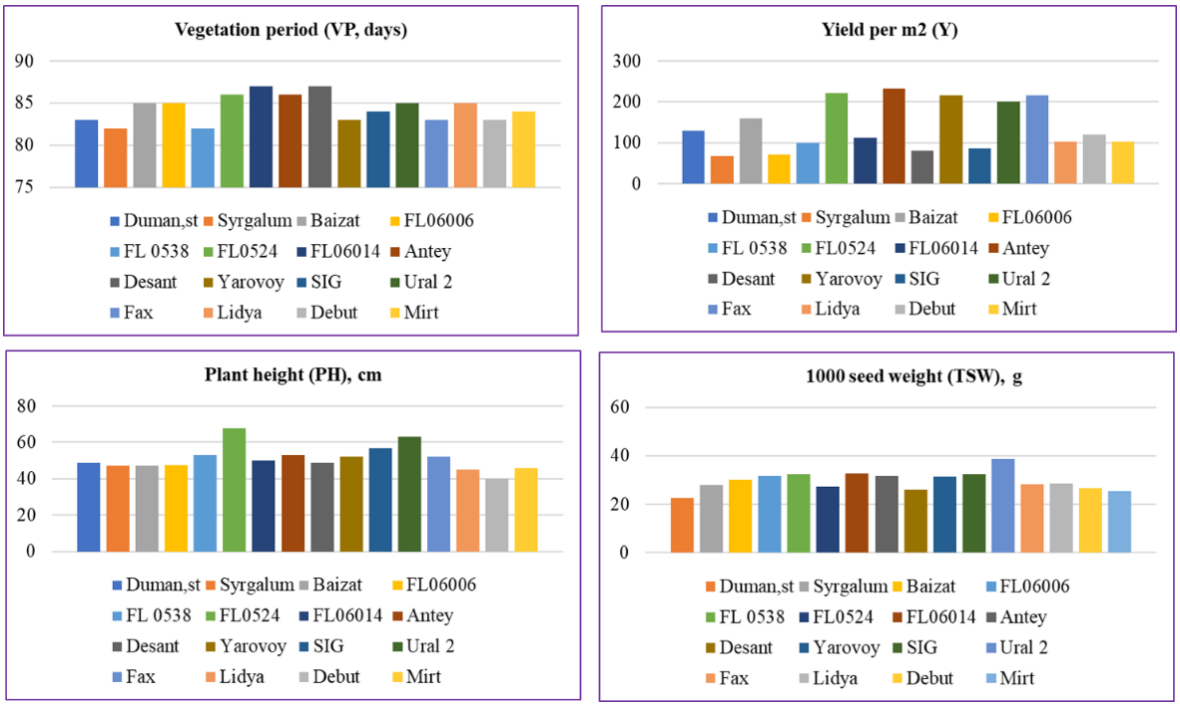

Figure 2. Variation of the genotypes based on the results of the test by the valuable agronomic traits in field conditions

The study of the vegetation period is essential for understanding the life cycle of oat plants, determining optimal conditions for cultivation, and predicting crop yield. Information about vegetation period duration and other characteristics helps to plan crop rotations, select varieties and plant species well-adapted to specific climatic conditions, and effectively use resources in plant selection programmes aimed at crop yield increase. Thus, recording individual phenophases and interphase periods of the oat varieties allowed selecting the earliest-ripening varieties adapted for the conditions of the Akmola region: Syrgalum, FL 0538, Yarovoy, Fax, and Debut. These varieties matured earlier than the standard or showed the same results (82-83 days).

Experimental data obtained in the course of our research evaluate the average value of grain yield and reveal the most productive genotypes cultivated in the northern region of Kazakhstan and formed in the contrasting climatic conditions. On average for two years, grain productivity of the varieties Yarovoy, Antey, Ural 2, and Fax was 200–232 g/m^2, which is higher than the standard variety by 102 g/m2.

Evaluation of structural elements of the oat genotypes. For all years of the research, productive tilleringgave a stable result of 1.8-1.9 pcs/1 plant. The FL06006 sample excelled and showed 1.8 pcs/1 plant for this trait. The trait of grain weight per plant had the most significant difference by year. Thus, the observations on the experimental plots showed fluctuations in the range of 0.7-1.84 g, where the varieties Fax and Ante excelled. According to the data from the experimental plots of the A.I. Barayev Research and Production Centre for Grain Farming, grain weight from the main panicle did not differ that much (0.49-1.24 g), while the number of grains in the panicle was from 15 to 44 pieces. The SIG, Yarovoy, and Fax varieties contained the most grains (34–44 pieces) (Table 2).

Table 2

Results of the productivity elements analysis of the selected oat varieties

Variety samples	Plant height, cm	Productive tillering, pc/1 plant	Grain weight per plant, g	Grain weight per panicle, g	Panicle length, cm	Number of grains in panicle, pc	Weight of 1000 grains, g
Duman, st	48,8	1,6	-	0,52	14,8	32	22,5
Syrgalum	47,1	1,6	0,8	0,54	15,3	20	27,9
Baizat	47	1,2	0,99	0,81	14,2	28	30,1
FL06006	47,6	1,8	0,73	0,65	14,1	20	31,8
FL 0538	53	1,5	0,79	0,49	17,5	15	32,3

Continuation of Table 2

Variety samples	Plant height, cm	Productive tillering, pc/1 plant	Grain weight per plant, g	Grain weight per panicle, g	Panicle length, cm	Number of grains in panicle, pc	Weight of 1000 grains, g
FL0524	68	1,1	1,09	0,79	16,1	29	27,2
FL06014	50	1,6	0,83	0,56	13,5	17	32,7
Antey	53	1,2	1,84	0,98	15,7	31	31,7
Desant	49	0,9	0,72	0,62	12,8	24	26,1
Yarovoy	52	1,1	1,34	1,07	12,8	34	31,5
SIG	57	1	1,17	1,11	17,4	34	32,5
Ural 2	63	1,4	0,88	0,8	16,7	21	38,6
Fax	52	1,5	1,62	1,24	14,8	44	28,2
Lidya	45	1,6	0,7	0,65	13,5	23	28,6
Debut	40	1,5	0,82	0,52	11,9	19	26,7
Mirt	46	1,3	1,18	0,7	12,8	27	25,5

Grain size shows the food significance of a variety, determines its nutrient reserve, germination capability, and food and fodder qualities of a genotype. At the same time, it is limited by the varietal characteristics of the plant and the duration of its development, i.e., varietal specificity in combination with environmental conditions. Lack of productive moisture and high temperatures during the grain filling significantly decrease grain size [11]. Consequently, the need to determine the adaptive potential of the shared oat gene pool on the trait of weight of 1000 grains by experimentation in different conditions is a relevant task for breeding. During the research the most favourable conditions for large grains formation were in 2023: weight of 1000 grains for the Baizat, FL06006, FL 0538, FL06014, Antey, Yarovoy, SIG and Ural 2 varieties was between 30.1 and 38.6 g, that was by 7.6–18.6 g higher than the same metric of the Duman standard variety.

Analysis of correlations between the research results. The success of crop breeding depends on genotypically determined correlations of qualitative traits, selection of parental forms for hybridisation, accurate and objective evaluation and rejection of plant breeding material [12]. Correlation was considered particularly relevant in cereal crop breeding at certain stages of the breeding process due to significant genotypes and a lack of seed material. Also, some unique characteristics of grain quality indicators are essential for plant breeding, such as the extent and variability of the relationship between individual traits and weather conditions in different years. Therefore, plant breeders face the challenge of identifying traits that overlap or have significant differences in the values of the studied indicators. If the absolute value of the correlation coefficient is large enough or close to linear dependence, it is also effective to use the correlation coefficients in the selection process [13, 14]. When sorting by a set of quality indicators, decisions must be made on various models that combine several indicators. To avoid selection bias, plant breeders should focus on critical quality and yield indicators that remain constant yearly and are closely related to other indicators. Thus, correlation analysis aims to identify quantitative traits of common oat genotypes relevant for selection improvement and to identify the best varietal samples for further crop breeding to improve grain quality and yield.

In this regard, PCA analysis was carried out to determine the relationships between the valuable traits of the oat genotypes. The results of PCA analysis demonstrated the degree of relative proximity and distance between the genotypes (Fig. 3).

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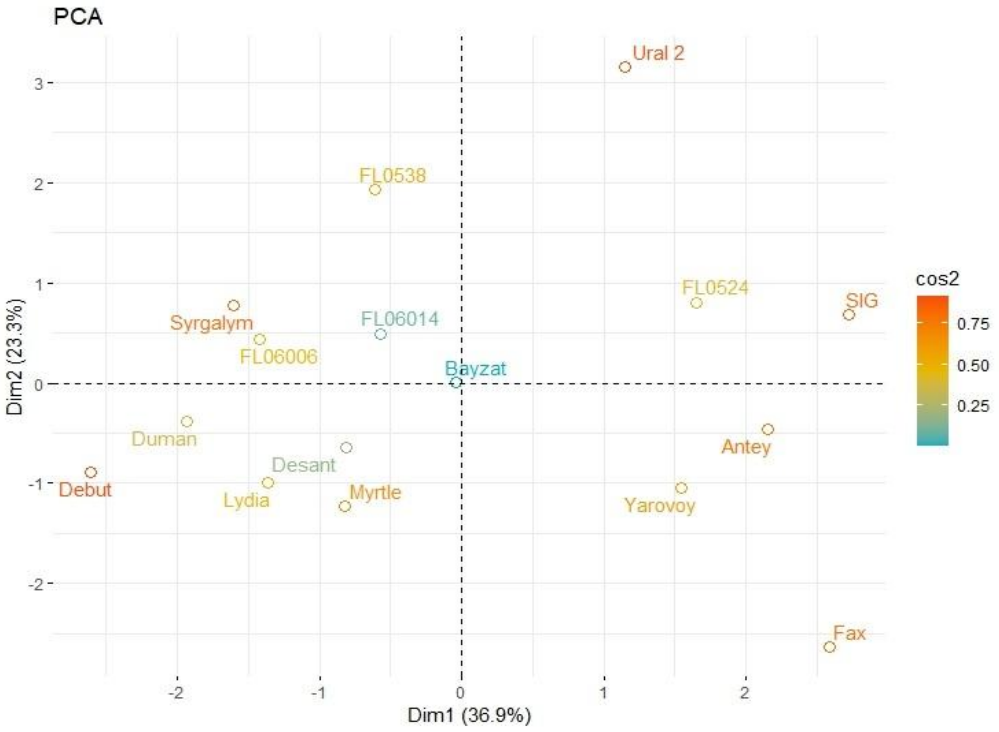

Figure 3. Results of PCA analysis of the yield-determining traits of the oat collection variety samples

The Dim1 and Dim2 axes are the main components: Dim1 (36.9 %) explains almost 37 % of the total variability in the data. Dim2 (23.3 %) explains another 23 %. Together, they provide about 60 % of the information on agronomic differences between the varieties. Each point represents a variety projected onto a new feature axis. The dots' colour reflects the array's contribution (cos2): the lighter the colour (closer to red), the more the variety influences the distribution. Debut, Syrgalum, Duman, and Lidya are on the left side of the graph. These varieties have similar characteristics, although some parameters may have lower values (e.g. weight of 1000 grains, panicle length). Debut stood out, occupying an extreme position in Dim1 and Dim2. Baizat and FL06014 are located near the centre: their characteristics are close to the average sample, i.e. they are "balanced". Antey, Yarovoy, Fax, SIG, and Ural 2 are situated on the right side: these varieties have distinct differences, especially Fax and Ural 2. Fax is highly distorted in both components, probably due to its maximum number of grains per panicle and the length of the plants. Thus, the varieties Fax, Ural 2 and Antey are unique and may be of interest for oat breeding, especially by yield characteristics. Due to its location near the centre, Baizat and FL06014 can be versatile and tolerate different conditions. The Debut and Lidya varieties, shifted to the left side of the graph, may have low values of the valuable agronomic traits.

Regression analysis determined the relationships between the individual traits (Fig. 4).

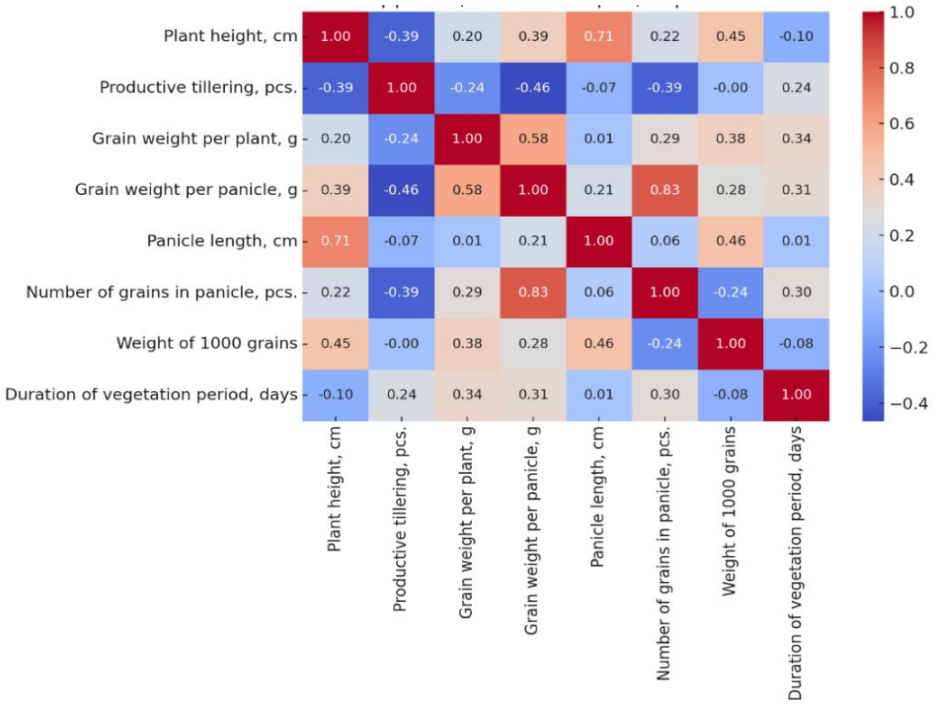

Figure 4. Matrix of the correlation traits of the oat genotypes

Correlation analysis revealed that plant height was moderately correlated with panicle length (r=0.71) and weight of 1000 grains (r=0.45). Number of grains in panicle correlated with seed weight per panicle (r=0.83), and seed weight per plant associated with the traits of seed weight per panicle (r=0.58), weight of 1000 grains (r=0.38) and with duration of vegetation period (r=0.34). A similar stable relationship was found between panicle seed weight and plant height (r=0.39), seed weight per plant (r=0.58) and number of grains per panicle (r=0.83). Other valuable traits, such as the number of grains in a panicle, are closely correlated with grain weight per panicle (r=0.83).

The following traits were negatively correlated: productive tillering and plant height; number of grains in panicle and productive tillering, grain weight per plant and number of grainsin panicle (r= -0.39-0.46). Conventional cluster analysis was used to group genotypes differing in valuable agronomic traits (Fig. 5).

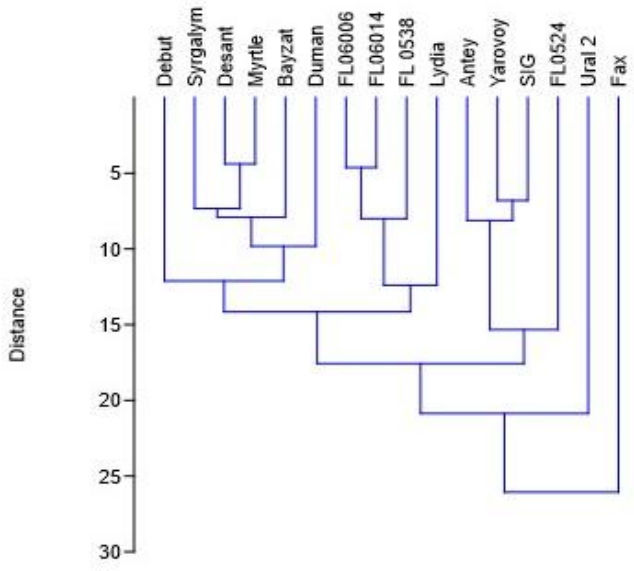

Figure 5. Grouping of the oat genotypes by the valuable traits

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Closely related varieties, such as Syrgalum and Desant, FL06014, and FL 0538, have similar characteristics. The most unique (distantly related) varieties are Fax, Ural 2, and FG524, as they are related to others at a long distance (about 25–30).

Thus, after determining the relationships between the valuable agronomic traits of the oat genotypes, it was found that the trait of 1000 grains is positively related to grain weight in panicle and duration of the vegetation period. The obtained results present valuable information for use in the oat breeding process.

Conclusions

Plant breeding on oat at the experimental plots of the A.I. Barayev Research and Production Centre for Grain Farming has selected several valuable genotypes, and various activation methods have increased the efficiency of the crop breeding process. The presented scientific work summarises the phenological characteristics of the varieties and shows the optimal ripening dates for the Syrgalum, FL 0538, Yarovoy, Fax, and Debut variety samples.

According to the results of the productivity evaluation of the genotypes, it can be concluded that some oat varieties are high-yielding. This is confirmed by the performance of the Yarovoy, Antey, Ural 2, Fax genotypes (200–232 g/m2). Highly productive tillering has a positive effect on total yield. Regarding grain yield per panicle, which directly affects yield per unit area, the Yarovoy, Ural 2, and Fax varieties should be highlighted. Analysis of panicle length shows that the FL 0538, FL 0524, Ural 2, and SIG genotypes are characterised by long panicles (16.1-17.5 cm), which contributes to larger grains in the panicle. TheBaizat, Antey, Yarovoy, SIG, Ural 2 varieties, and the FL06006, FL 0538, and FL06014 samples have the most significant weight of 1000 seeds (30.1-38.6 g), which points to high quality and large size of grains. According to the results of correlation analysis, the most closely related pair of traits was identified: productive tillering and grain weight per panicle (r=0.46), which can be effective in oat breeding to achieve high yield.

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Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Dyussibayeva E.N**. – Introduction; Experimental, Results and Discussion, Conclusions; **Dolinny Y.Y**. – Introduction, Experimental, Results and Discussion; Conclusions; **Zhirnova I.A**. – Experimental, Results and Discussion; **Rysbekova A.B**. – Introduction, Experimental, Results and Discussion; Conclusions; **Zeinullina A.E**. – Experimental, Results and Discussion; **Orazov A.E**. – Introduction, Statistical processing of the obtained data; Conclusions; **Izbastina K.S**. – Introduction, Experimental, Results and Discussion; **Abylkairova M.M**. – Introduction, Results and Discussion; Conclusions.

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Ақмола облысы жағдайындағы егістік сұлы генотиптерінің селекциялық белгілері арасындағы корреляцияны зерттеу

Зерттеудің мақсаты ауылшаруашылық өсімдіктерінің өнімділігін арттыруға бағытталған Селекциялық бағдарламалар үшін маңызды сұлы тұқымының (Avenasativa) құнды агрономиялық белгілері арасындағы корреляциялық байланыстарды қарастыру. Зерттеу 2023-2024 жылдары А.И. Бараев атындағы Астық шаруашылығы ғылыми-өндірістік орталығының далалық тәжірибелік учаскелерінде жүргізілді. Бақылаулар өсімдік биіктігінің биометриялық өлшемдері мен өнімділіктің морфологиялық белгілерін қолдану арқылы жүзеге асырылды. Биологиялық материал ретінде сұлы 16 генотиптерден жиналды. Нәтижелер корреляция коэффициенттері, дисперсиялық талдау, бірнеше регрессия және белгілер арасындағы қатынастарды зерттеу арқылы талданды. Өнім сабағының шашақгүліндегі дәндердің массасы мен санын қоспағанда (r = -0,39 0 0,46), бірқатар белгілерге сызықтық тәуелділігі бар екендігі анықталды. Корреляциялық талдау нәтижелері бойынша келесі қатынастар анықталды: 1000 дәннің массасы және өсімдіктің биіктігі; өсімдіктен тұқымның массасы және шашақгүлінің ұзындығы (r = 0,38 0 0,46); өнімнің сабағы мен шашақгүліндегі тұқымның массасы (r = 0,46). Бұл белгілерді сұлының жоғары өнімді түрлерін алу үшін селекциялық процесте тиімді пайдалануға болады. Өнімділік элементтерін құрылымдық талдау нәтижесінде құнды белгілер кешені бар генотиптер анықталды, олар: көктемгі және Факс сорттары, сондай-ақ FL 0524 үлгісі. Осы ген түрлері сұлы өсімдіктерінің өнімділігін арттыру үшін көздер ретінде ұсынылады.

Кілт сөздер: сұлы, корреляция, селекция, сорт, шаруашылық-құнды белгілер, вегетациялық кезең, өнімділік.

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Изучение корреляционных связей между селекционными признаками генотипов овса посевного в Акмолинской области

Целью настоящего исследования было изучение корреляционных связей между ценными агрономическими признаками овса посевного (*Avena sativa*), важными для селекционных программ, направленных на повышение продуктивности сельскохозяйственных растений. Исследования проводились в

2023–2024 гг. на полевых опытных участках Научно-производственного центра зернового хозяйства им. А. И. Бараева. Наблюдения осуществлялись с использованием биометрических измерений высоты растений и морфологических признаков продуктивности. Биологическим материалом послужили 16 генотипов овса. Результаты анализировались с помощью коэффициентов корреляции, дисперсионного анализа, множественной регрессии и изучения взаимосвязей между признаками. Установлено, что продуктивная кустистость имеет линейную зависимость с рядом признаков, за исключением массы и числа зерен в метелке (r = –0,39…0,46). По результатам корреляционного анализа выявлены следующие взаимосвязи: масса 1000 зерен и высота растения; масса семян с растения и длина метелки (r = 0,38…0,46); продуктивная кустистость и масса семян с метелки (r = 0,46). Эти признаки могут быть эффективно использованы в селекционном процессе для получения высокоурожайных форм овса. В результате структурного анализа элементов продуктивности были выделены генотипы, обладающие комплексом ценных признаков: сорта Яровой и Факс, а также образец FL 0524. Эти генотипы рекомендованы в качестве источников для повышения продуктивности растений овса.

*Ключевые слова***:** овес, корреляция, селекция, сорт, хозяйственно-ценные признаки, вегетационный период, урожайность.

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Species and functional diversity of forest communities with wild fruits plants in Central Kazakhstan

The alpha-, beta-, gamma-, and functional diversity of forest communities of Central Kazakhstan, which are habitats of wild fruit plants, were analysed. Forest communities were studied in seven sites represented by mountain forests, island forests, and steppe kolki. A total of 41 relevés were collected and analysed. We counted 195 species of vascular plants, including 10 species of fruit plants: *Crataegus sanguinea* Pall., *Lonicera tatarica* L., *Ribes aciculare* Sm., *Ribes nigrum* L., *Ribes saxatile* Pall., *Rosa acicularis* Lindl., *Rosa laxa* Retz., *Rosa majalis* Herrm., and *Rosa spinosissima* L. Results of non-metric multidimensional scaling revealed no dividing of relevés between the studied sites. Cluster analysis using Ward's method identified 4 groups of relevés. No statistically significant differences in alpha-diversity indices were found between the groups. Beta-diversity assessment based on Jaccard distance showed that the groups differed well in species composition. The results of functional diversity analysis based on ecological-coenotic groups and the calculation of indicator species values showed differences in the structure of the four community groups. It was shown that *Rosa majalis* is a significant indicator species for the mountain-forest massif of Karkaraly, and *Ribes nigrum* — for steppe kolki with predominance of forest species. Other fruit species were found in all analysed groups of communities and did not show specific coenotic predilection.

Keywords: communities, biodiversity assessment, indVal, ecological-coenotic structure, GBIF.

Introduction

Forests in Central Kazakhstan cover less than 2 % of the area [1]. Forest habitats in this territory are represented by relatively small steppe kolki and larger mountain-forest areas confined to the lowlands of the Kazakh Shallow Forest (Sary-Arka) [2]. The mountain-forest areas located within the Ob-Irtysh interfluve are relicts of a formerly continuous forest territory connected with the forests of Western Siberia and Altai during the cold and wet Pleistocene epochs. Due to this, they have preserved a complex of boreal (and other forest) plant species considerably distant from the main range [3].

Forest communities in Central Kazakhstan are still poorly explored. The floristic diversity of some mountain-forest massifs has been studied [4–6]; a few assessments of species and functional diversity of forest communities are available [7]. At the same time, forest areas of Central Kazakhstan are key habitats for most species of wild fruit plants of the genera *Rosa*, *Ribes*, *Lonicera*, and *Crataegus* [8–9]. Studying the natural habitat of these species is relevant for assessing ecosystem services for the use of wild fruit plants as genetic, food and medicinal resources. The aim of the study was to assess the diversity and structure of forest communities with wild fruits in Central Kazakhstan.

Materials and methods

The climate of Central Kazakhstan is sharply continental, with hot, temperate summers and cold, snowy winters. The average temperature in winter is about –13 °C, in summer—about +24 °C, and annual precipitation is 180–250 mm [10]. Steppe and semi-desert vegetation prevails [11].

Field studies were carried out in 7 areas (Fig. 1) located in the Karagandy, Ulytau, and Akmola regions.

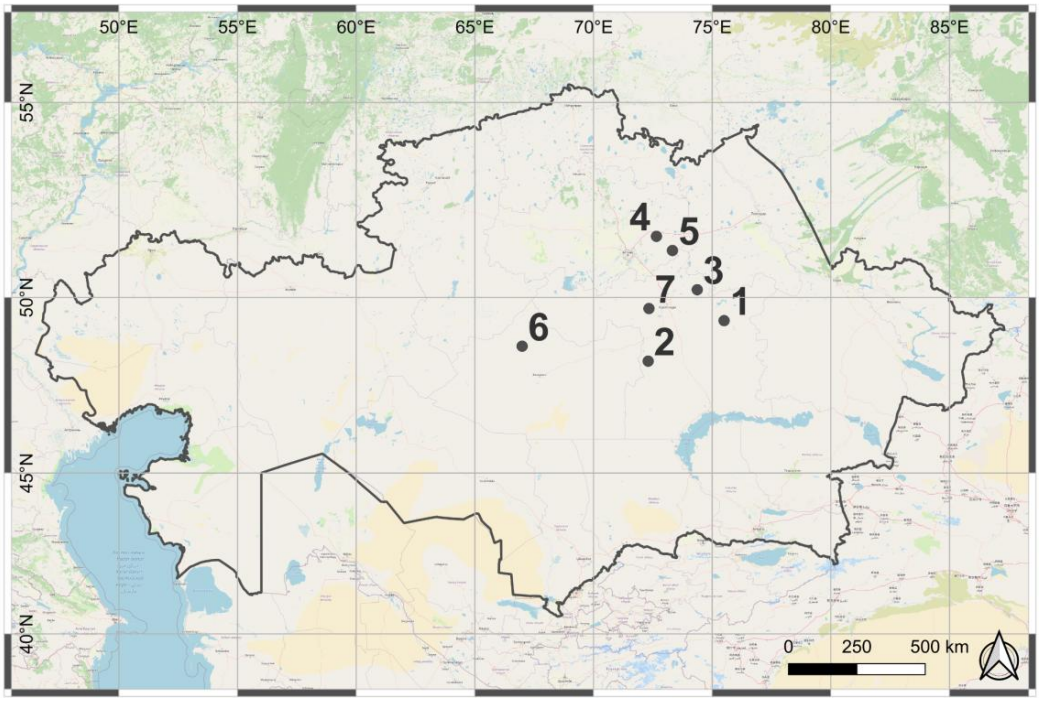

Figure 1. Study areas. 1 - Karkaraly National Park (mountain forests); 2 - Ortau mountain forests; 3 - island pine barrens near Kerney settlement; 4 and 5 - Buiratau National Park: 4 - steppe kolki near Ereimentau town; 5 - steppe kolki near Belodymovka village; 6 - steppe kolki Ulytau National Park; 7 - steppe kolki near Koyandy hill.

We studied 41 vegetation plots of 100 m^2 following the standard relevé method [12]. All vascular plants and their abundance according to the Braun-Blanquet cover-abundance scale were recorded in herb, shrub, and tree layers. In Karkaraly, 17 vegetation relevés were performed: 7 in Ortau, 4 near Kerney, 3 near Ereimentau, 4 near Belodymovka, 4 in Ulytau, and 2 near Koyandy. Most of the studied tree stands were small-leaved (*Betula pendula* Roth and *Populus tremula* L.)*;* 3 sample plots were described in pine forests (*Pinus sylvestris* L.), and 2—in black alder forests (*Alnus glutinosa* (L.) Gaertn.). All data collected were digitised, standardised according to the Darwin Core [13] and published through the Global Biodiversity Information Facility (GBIF) portal [14].

Data analysis was performed using the R environment [15]. At the first step to visualise the gradient of vascular plant species composition across the study areas, we used non-metric multidimensional scaling (NMDS) calculated in the vegan package, *metaMDS()* function [16]. After that, Ward's cluster analysis with Hellinger distance was performed for grouping of relevés (*vegdist()* and *hclust()* functions). For each group, we assessed alpha, beta, and gamma diversity. Alpha diversity scores were calculated as species richness, the Shannon index, and the Simpson index [17]. The significance of differences in alpha diversity scores between groups was assessed using one-way ANOVA (*aov()* function). Group-level species beta-diversity was measured using the Jaccard's distance. Gamma diversity was estimated as the total number of species in each group [18]. Functional diversity was estimated based on the diversity of ecological-coenotic groups (ECG) [19]. The ECGs published in [20] were used; calculations were made taking into account species abundance. For each group, indicator species values were calculated using the Indval.g algorithm implemented in the *multipatt()* function of the Indicspecies package [21].

Results and Discussion

A total of 195 vascular plant species were recorded in our relevés. The following species of wild fruit plants were counted: *Crataegus sanguinea* Pall., *Lonicera tatarica* L., *Ribes aciculare* Sm., *Ribes nigrum* L., *Ribes saxatile* Pall., *Rosa acicularis* Lindl., *Rosa laxa* Retz., *Rosa majalis* Herrm., and *Rosa spinosissima* L.

At the level of 7 study areas, the NMDS analysis did not result in a clear division of vegetation relevés (Fig. 2). Relevés from the Karkaraly and Ortau mountain forests and black-alder stands from the Belodymovka were placed in the left part of the ordination diagram. Island pine barrens and steppe kolki were in the right part of the diagram.

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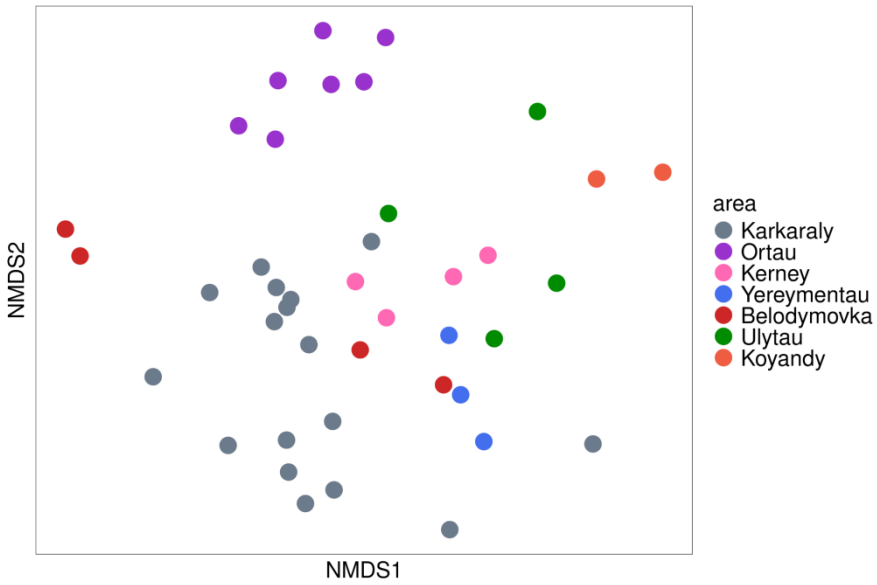


Figure 2. NMDS ordination of 41 vegetation relevés from forests of Central Kazakhstan.

As a result of cluster analysis, 4 groups of relevés were divided (Fig. 3). Cluster 1 grouped most of the relevés from Karkaraly mountain forests. Cluster 2 was composed of the relevés in steppe kolki described in Yereymentau, Belodymovka, Ulytau, and Karkaraly. Cluster 3 was related to black-alder stands near the Belodymovka study area. Cluster 4 grouped relevés belonging to the Ortau mountain forests, island pine barrens near Kerney, and steppe kolki from Koyandy and Ulytau.

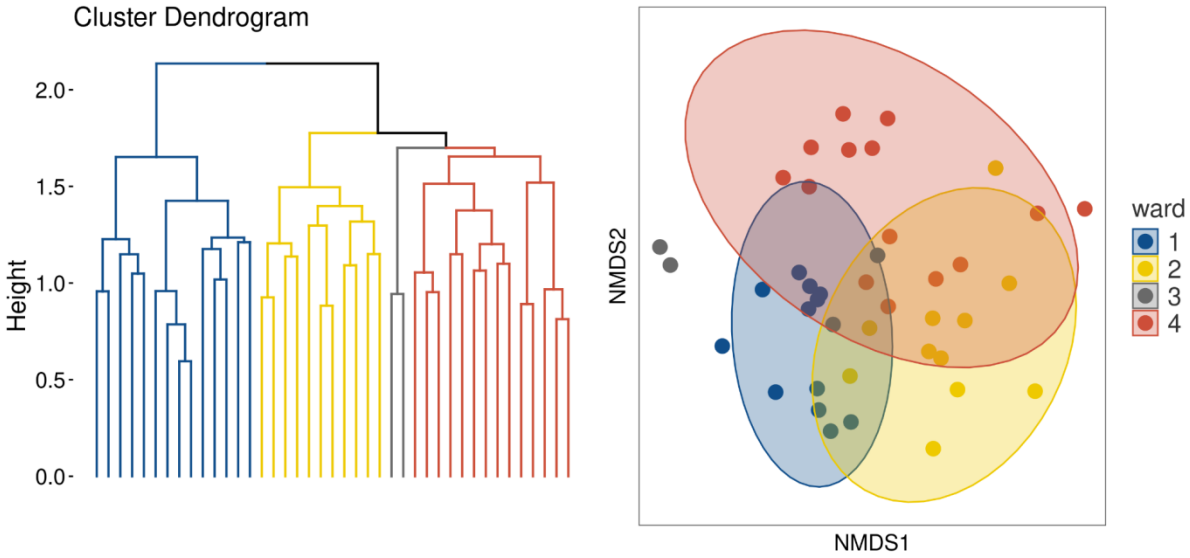
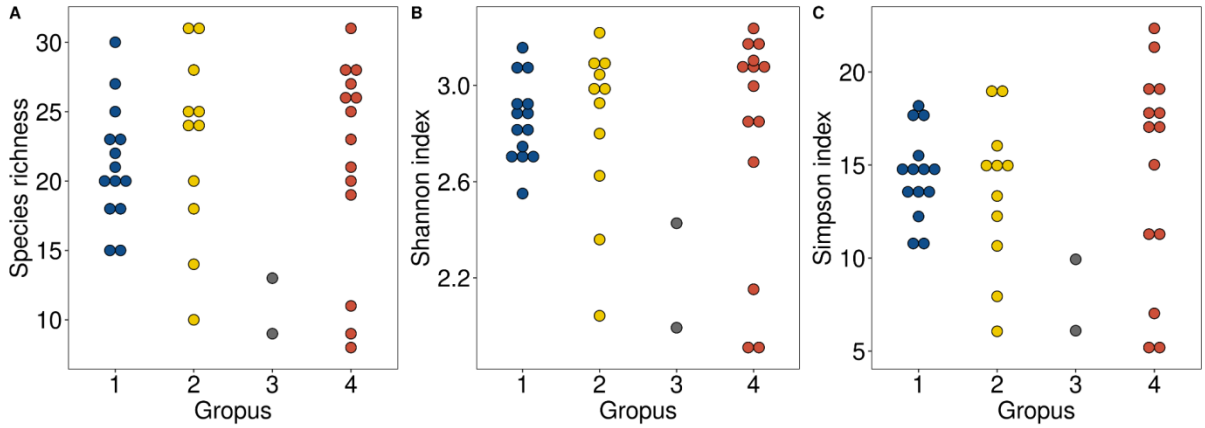


Figure 3. Results of cluster analysis of 41 vegetation relevés from forests of Central Kazakhstan. Cluster diagram (left) and clusters in the NMDS diagram (right)

No significant differences in alpha diversity scores were found between groups 1, 2, and 4 ($P>0.05$, Fig. 4). Overall, group 1 had narrower ranges in species richness, Shannon, and Simpson indexes than groups 2 and 4. Group 3 was not included in the comparisons due to the small sample size (only 2 relevés). More relevés are needed to characterise this group. However, black alder forests are a rare community type in Central Kazakhstan, and *Alnus glutinosa* is a protected tree species [22]. The total number of species (gamma diversity) was 108 in Group 1, 112 in Group 2, 16 in Group 3, and 114 in Group 4.



The results of the beta-diversity assessments found differences in species composition between groups 1, 2, and 4 (Table 1). The large distances of Group 3 with other groups may be related both to insufficient sample size and to the specific species composition of black-alder forests.

Table 1

Jaccard distances for divided vegetation groups

	Group 1	Group 2	Group 3	Group 4
Group 1	0			
Group 2	0.63	0		
Group 3	0.92	0.93	0	
Group 4	0.64	0.57	0.92	0

The results of the functional diversity assessment showed that the analysed groups of relevés differed in the composition and abundance of ecological-coenotic groups.

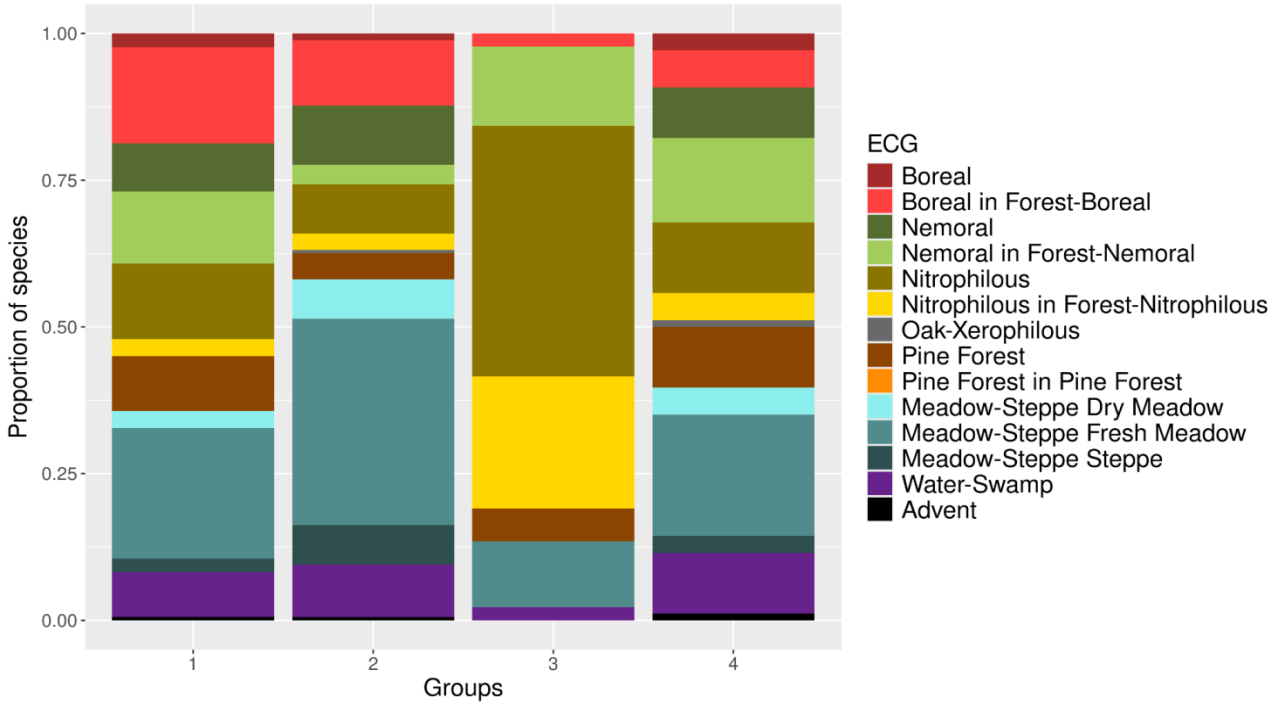


Figure 4. Diversity of ecological and coenotic groups in forests of Central Kazakhstan.
For a detailed description of the groups, see [20].

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The structure of forest communities in Group 1 (Karkaraly mountain forests) is dominated by forest-related species, with considerable participation of boreal species. This result confirms the evidence of the long presence of forests in this area [3]. Nitrophilous species were expectedly dominant in the ECG structure of group 3 (black alder forests), and the participation of steppe species was minimal. Relevés of steppe kolki were divided into two groups that differ in the ECG structure. Steppe species predominate in Group 2, and boreal and nemoral species predominate among forest species. In group 4, the participation of forest species is higher than that of steppe species. The most represented are nemoral species; compared to group 2, the participation of pine-forest species is higher. The reasons for these differences require further research. It is likely that differences in the structure of ECGs may be related to the area and age of steppe stakes, pasture load, or fire frequency.

The results of calculating indicator species in the analysed groups agree well with the estimates of functional diversity (Table 2). Most of the significant indicators for Group 1 are represented by forest species. In Group 2, all indicator species were steppe ECG. In Group 3, two of the three significant indicators were nitrophilous forest species. In Group 4, both forest and steppe species were indicator species.

Table 2

Indicator species values for analysed groups of relevés

species	ECG code	Indval.g value	p-value
Group 1			
Clematis sibirica (L.) Mill.	Boreal	0.535	0.030
Rosa majalis Herrm.	Nemoral	0.675	0.015
Equisetum pratense Ehrh.	Nemoral In Forest-Nemoral	0.661	0.005
Prunus padus L.	Nitrophilous In Forest-Nitrophilous	0.711	0.005
Geum rivale L.	Nitrophilous In Forest-Nitrophilous	0.535	0.025
Potentilla argentea L.	PineForest	0.823	0.005
Veronica pinnata L.	PineForest	0.655	0.005
Vicia sepium L.	Meadow-Steppe FreshMeadow	0.655	0.005
Ligularia spp.	Meadow-Steppe FreshMeadow	0.607	0.015
Thalictrum foetidum L.	Meadow-Steppe DryMeadow	0.580	0.030
Group 2			
Plantago maxima Juss. ex Jacq.	Meadow-Steppe FreshMeadow	0.853	0.005
Scrophularia nodosa L.	Meadow-Steppe FreshMeadow	0.618	0.010
Achillea millefolium L.	Meadow-Steppe FreshMeadow	0.558	0.050
Artemisia dracunculus L.	Meadow-Steppe DryMeadow	0.522	0.030
Filipendula vulgaris Moench	Meadow-Steppe Steppe	0.624	0.010
Group 3			
Humulus lupulus L.	Nitrophillous	0.816	0.01
Cardamine amara L.	Nitrophilous InForest-Nitrophilous	1.000	0.01
Galeopsis bifida Boenn.	Meadow-Steppe FreshMeadow	0.707	0.01
Group 4			
Senecio jacobaea Loscos & Pardo	Nemoral	0.593	0.025
Poa nemoralis L.	Nemoral InForest-Nemoral	0.683	0.035
Ribes nigrum L.	Nitrophillous	0.612	0.030
Pentanema britannicum (L.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M. Mart.Ort.	Nitrophillous	0.595	0.045
Calamagrostis epigejos (L.) Roth	PineForest	0.628	0.025
Cuscuta europaea L.	Meadow-Steppe FreshMeadow	0.598	0.010
Crepis tectorum L.	Meadow-Steppe DryMeadow	0.756	0.005
Turritis glabra L.	Meadow-Steppe DryMeadow	0.566	0.025

It should also be noted that among the significant indicator species, 2 fruiting plants were recorded: *Rosa majalis* for Group 1 and *Ribes nigrum* for Group 4. This result shows that these species have specific requirements for habitat conditions, unlike other fruiting species that occurred in different community groups.

Conclusions

Our study provides, for the first time, quantitative forest biodiversity assessments in Central Kazakhstan. We found that the studied forests are diverse in species diversity and composition of the herbaceous layer. Using quantitative analysis methods, 4 groups of forest communities were obtained: (1) forests of the Karkaraly mountain-forest massif dominated by forest species; (2) black alder forests dominated by nitrophilous species; (3) steppe kolki dominated by steppe species; (4) steppe kolki dominated by forest species. The identified groups do not differ in alpha diversity metrics but differ significantly in species composition. It is also found that most of the fruiting species are found in all community groups. Strict cenotic confinement is shown for *Rosa majalis* (Karkaraly mountain forests) and *Ribes nigrum* (kolki dominated by forest species). These species were significant indicators in corresponding groups of forest communities.

Obtained results are relevant for prognosing potential habitats for wild fruit plants. Vegetation relevés will be used for further studies of forest biodiversity in the Karkaraly State National Nature Park. Primary data available through GBIF contributes to filling gaps in Kazakhstan's digital biodiversity map.

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Author contributions

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Орталық Қазақстандағы жабайы жеміс өсімдіктері бар орман қауымдастықтарының түрлік және құрылымдық әртүрлілігі

Жұмыста жабайы жеміс өсімдіктерінің мекендейтін жері болып табылатын Орталық Қазақстанның орман қауымдастықтарының альфа-, бета-, гамма- және құрылымдық әртүрлілігіне талдау жүргізілді. Орман қауымдастықтары жеті аумақта зерттелді, олардың қатарына таулы-орман массивтері, аралдық қарағайлы ормандар және далалық тоғай алқаптары кіреді. Барлығы 41 сипаттама талданды. Барлық сипаттамалар бойынша 195 тамырлы өсімдік түрі тіркелді, олардың ішінде 10 жабайы жемісті түрлер: *Crataegus sanguinea* Pall., *Lonicera tatarica* L., *Ribes aciculare* Sm., *Ribes nigrum* L., *Ribes saxatile* Pall., *Rosa acicularis* Lindl., *Rosa laxa* Retz., *Rosa majalis* Herrm., және *Rosa spinosissima* L. NMDS зерттелген учаскелердің сипаттамалары арасында айқын бөліну болмағанын көрсетті. Вард әдісімен жүргізілген кластерлік талдау негізінде төрт топ анықталды. Бұл топтар арасында альфа-әртүрлілік бағалау көрсеткіштері бойынша статистикалық тұрғыдан маңызды айырмашылықтар анықталмады. Жаккард қашықтығы бойынша есептелген бета-әртүрлілік нәтижелері анықталған топтардың түрлік құрамы тұрғысынан жақсы ерекшеленетінін көрсетті. Қауымдастық құрылымының әртүрлілігі эколого-ценоздық топтар мен индикаторлық түрлерді талдау арқылы бағаланды. Нәтижесінде төрт қауымдастық тобының құрылымында айырмашылықтар бар екені анықталды. *Rosa majalis* Қарқаралы таулы-орман алқаптарына тән индикаторлық түр ретінде ерекшеленсе, *Ribes nigrum* орманды түрлер басым дала тоғайларына тән түр болып табылды. Қалған жемісті түрлер барлық зерттелген қауымдастық топтарында кездесіп, айқын ценоздық бейімділік көрсетпеді.

Кілт сөздер: қауымдастықтар, биологиялық әртүрлілікті бағалау, indVal, экологиялық-ценотикалық құрылым, GBIF.

Н.В. Иванова, М.П. Шашков

Видовое и структурное разнообразие лесных сообществ Центрального Казахстана с участием дикорастущих плодовых

В работе проведен анализ альфа-, бета-, гамма- и структурного разнообразия лесных сообществ Центрального Казахстана, являющихся местообитаниями диких плодовых растений. Лесные сообщества исследованы на семи участках, представленных горно-лесными массивами, островными борами и степными колками. Всего проанализировано 41 описание. Во всем массиве описаний учтено 195 видов сосудистых растений, в том числе 10 видов плодовых: *Crataegus sanguinea* Pall., *Lonicera tatarica* L., *Ribes aciculare* Sm., *Ribes nigrum* L., *Ribes saxatile* Pall., *Rosa acicularis* Lindl., *Rosa laxa* Retz., *Rosa majalis* Herrm., и *Rosa spinosissima* L. Результаты неметрического многомерного шкалирования не выявили разделения описаний между исследованными участками. При помощи кластерного анализа методом Варда выделено 4 группы описаний. Между группами не выявлено статистически значимых различий по показателям альфа-разнообразия. Оценка бета-разнообразия на основе расстояния Жаккара показала, что выделенные группы хорошо различаются по видовому составу. Результаты анализа структурного разнообразия на основе эколого-ценотических групп и расчета индикаторных видов показали различия в структуре четырех групп сообществ. Показано, что *Rosa majalis* является значимым индикаторным видом для горно-лесного массива Каркаралы, а *Ribes nigrum* — для степных колков с преобладанием лесных видов. Остальные виды плодовых встречались во всеханализируемых группах сообществ и не показали специфической ценотической приуроченности.

Ключевые слова: сообщества, оценки биоразнообразия, indVal, эколого-ценотическая структура, GBIF.

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Comparative anatomic analysis of leaves of *Lonicera tatarica* and *Lonicera microphylla*

The study of anatomical structure of vegetative organs of plants allows to estimate biological and ecological features of species and to reveal features of structure, which allow to carry out identification of taxa at microscopic level. This is especially important for taxonomically close species. The paper presents the results of a comparative anatomical study of leaves of *Lonicera tatarica* and *Lonicera microphylla*. Leaf samples were collected in the summer period of 2024, fixed in Strauss-Fleming's solution, surface preparations and transverse sections were made manually. The results of the studies allowed us to establish that both honeysuckle species are characterized by light leaves, of dorso-ventral type with differentiated mesophyll into columnar and spongy tissues. Stomata are few, of anomocytic type, localized mainly on the underside of the leaf. In both species, small rounded druzes of calcium oxalate are present. Differences between the species were found in the shape of leaf epidermal cells, the number of columnar and spongy tissue layers, the shape of conductive bundles, the number of calcium oxalate druses and the presence of simple trichomes.

Keywords: *Lonicera tatarica*, *Lonicera microphylla*, anatomical structure, leaf, petiole, comparative study.

Introduction

Nowadays, the study of fruit plants is of great importance as sources of food, vitamins and biologically active substances [1, 2]. In Karaganda region, the genus *Lonicera* L. (family Caprifoliaceae Juss.) is a promising object for use as a fruit crop and object for green building.

Anatomo-morphological structure of plants in the comparative aspect is an indicator of biological features and ecology of species, and also allows to establish affinity between taxa [3, 4], to act as diagnostic signs of medicinal plant raw materials [5, 6].

In Karaganda region *Lonicera tatarica* L. and *Lonicera microphylla* Willd. ex Schult are of interest as ornamental, food and medicinal plants. Thus, *Lonicera tatarica* is characterized by resistance to climatic conditions, weakly damaged by diseases and pests, suitable for wide application in green building [7], during flowering as an excellent mellifer, berries contain a complex of vitamins and minerals [8], suitable in cooking, and weaving as a source of medicinal raw materials [9]. The fruits of *Lonicera microphylla* have antioxidant and tonic properties [10].

As a study of biological properties of both species of honeysuckle, the objective was to investigate the anatomical parameters of the leaf and to identify the diagnostic characters of the species.

Experimental

Leaf samples of both species of *Lonicera* L. (Fig. 1) were collected in the Karkaraly mountains (Karkaraly district, Karaganda region) in June 2024. Species identification was carried out at the Department of Botany of the University. The plant specimens were deposited in the herbarium fund of the Faculty of Biology and Geography of Karaganda Buketov University.

Dry leaves (leaves plates and petioles) were soaked in Strauss-Fleming mixture (distilled water: ethyl alcohol 96 % — glycerol 40 % in the ratio 1:1:1), transverse sections and surface preparations were prepared using a blade [11].

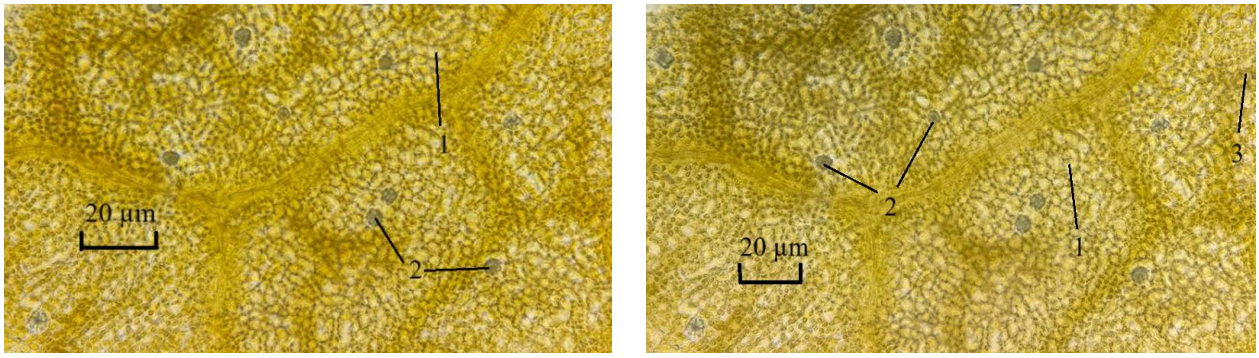
The description of micropreparations was carried out in accordance with the methodological guidelines [12, 13]. During description, attention was paid to cell structure, shape, presence of trichomes and inclusions, shape of stomata, shape and localization of conductive bundles.

A **B**

Figure 1. *Lonicera tatarica* and *Lonicera microphylla* in fruitening phase, Karkaraly Mountains

Results and Discussion

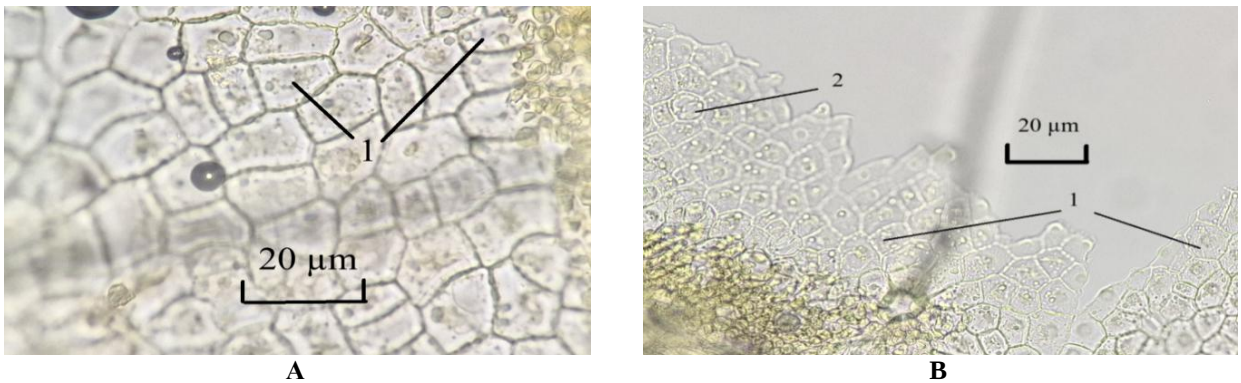
The cells of the upper and lower sides of the leaf of *L.tatarica* are rounded or slightly elongated (Fig. 2), the walls are slightly curved. Rounded druzes of calcium oxalate are translucent on both sides. Stomata are oval in shape, anomocytic type, localized mainly on the lower side of the leaf.



A **B**

Figure 2. Micropreparation of the leaf of *Lonicera tatarica*, fragments of preparations with surface:
A — upper epidermis, B — lower epidermis; 1 — epidermis cells, 2 — druses; 3 — stomata

Cells of upper and lower epidermis *of Lonicera microphylla* leaf are characterized by elongated or rounded shape, with straight and distinctly thickened walls (Fig. 3). Stomata are localized on the lower side of the leaf, rounded, anomocytic type. Trichomes are placed along the central vein; they are simple, 1-2-celled.



A **B**

Figure 3. Micropreparation of the leaf of *Lonicera microphylla*,
fragments of preparations with surface: 1 — epidermis cells, 2 — stomata

On the transverse section (Fig. 4) the leaf of *L. tatarica* is of dorso-ventral type, the mesophyll is indistinctly differentiated into palisade and spongy tissues. A single-layered epidermis is located on both sides. Its cells are rounded in shape, with a clearly visible layer of cuticle on the outer side. In the area of the main and lateral veins, areas of collenchyma are located under the epidermis, with mesophyll between the veins. Under the upper epidermis there are 1-2 layers of palisade tissue, on the lower side there are cells of spongy tissue with developed intercellular layers. Conductive bundles of elliptical shape, collateral, closed type, reinforced by sections of sclerenchyma. Numerous druzes of calcium oxalate are localized in the mesophyll.

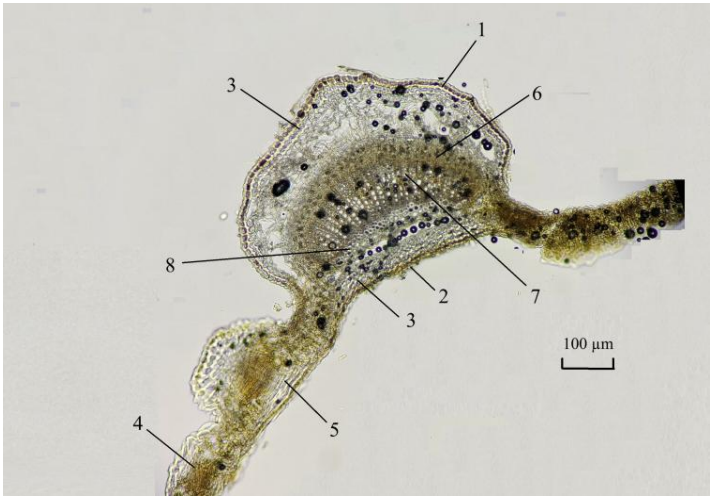


Figure 4. Transverse section of a leaf of *Lonicera tatarica*.
Fragment in the area of the central vein: 1 — lower epidermis, 2 — upper epidermis, 3 — collenchyma, 4 — spongy mesophyll, 5 — palisade mesophyll, 6 — phloem, 7 — xylem, 8 — sclerenchyma

The transverse section of *Lonicera microphylla* is also flat, dorso-ventral on the transverse section (Fig. 5), not clearly differentiated into palisade and spongy tissue. On both sides, the leaf is surrounded by a 1-layer epidermis composed of oval cells and a thick layer of cuticle. The columnar tissue is arranged in 2 layers and the spongy tissue in 2-3 layers. Conducting bundles are oval curved, collateral, closed type.

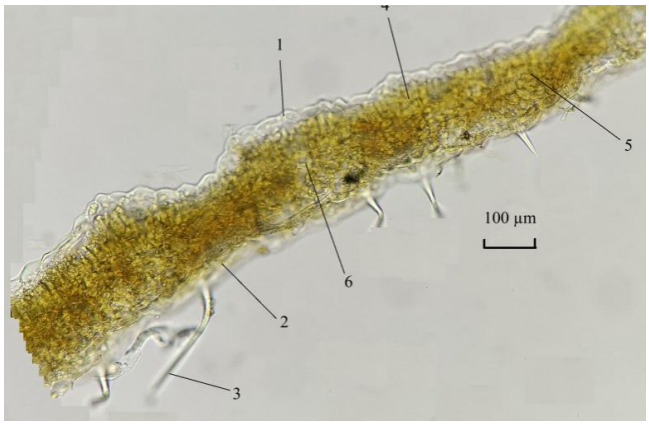


Figure 5. Transverse section of a leaf of *Lonicera microphylla*.
Fragment in the area of the central vein: 1 — lower epidermis, 2 — upper epidermis, 3 — collenchyma, 4 — spongy mesophyll, 5 — palisade mesophyll, 6 — phloem, 7 — xylem, 8 — sclerenchyma

The leaf petiole of Tatar honeysuckle is broadly ovate, slightly curved in cross section (Fig. 6). Along the perimeter there is a single-layer epidermis with a thick layer of cuticle. Beneath the epidermis lies a multilayered collenchyma. In the center of the petiole lies a vascular-fiber bundle, collateral, closed type, reinforced by sclerenchyma strands. The mesophyll zone is thin, composed of thin-walled parenchyma cells, with large intercellular cells and small druzes of calcium oxalate.

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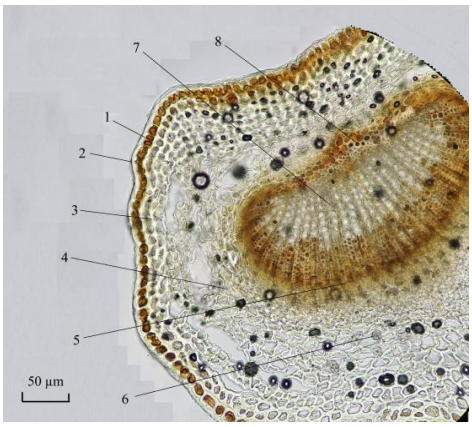

Figure 6. Transverse section of *Lonicera tatarica* leaf petiole: 1 — epidermis, 2 — cuticle, 3 — collenchyma, 4 — mesophyll, 5 — phloem, 6 — druzes, 7 — xylem, 8 — sclerenchyma

The leaf petiole of *Lonicera microphylla* on a transverse section is curved (Fig. 7). Rounded epidermal cells with cuticle, adjacent to 2–6-layer collenchyma, lie along the perimeter. The conducting bundle is narrow, elongated, collateral, of closed type, surrounded by sections of sclerenchyma. Numerous druzes of calcium oxalate are placed around the central vein under a layer of lamellar collenchyma. Few simple trichomes are noted.

Figure 7. Transverse section of a leaf of *Lonicera microphylla*. Fragment in the area of the central vein: 1 — epidermis, 2 — trichomes, 3 — collenchyma, 4 — calcium oxalate druses, 5 — central conductive bundle.

Comparison of anatomical features of the leaf of both species of honeysuckle allowed us to identify characteristic features (Table).

T a b l e

Comparative anatomical features of the leaf of *Lonicera tatarica* and *Lonicera microphylla*

Characteristics	*Lonicera tatarica*	*Lonicera microphylla*
Cells of leaf epidermis	Rounded or slightly elongated	Elongated or rounded
Drusae of the leaf lamina	+	-
Stomata	Anomocytic, localized on the lower side	Anomocytic, localized on the lower side
Trichomes on the leaf lamina	-	Simple
Type of leaf on transverse section	Dorso-ventral	Dorso-ventral
Mesophyll	Differentiated into palisade and spongy tissues	Differentiated into palisade and spongy tissues
Number of layers of columnar tissue	1-2	2
Number of layers of spongy tissue	2-3	2-3
Shape of conductive bundles	Elliptical	Oval
Shape of petiole on transverse section	Broadly ovate, slightly curved	Narrow-ovate, strongly curved
Druses	Few	Numerous

Thus, differences in the shape of leaf epidermis cells, presence of trichomes and druses of calcium oxalate, number of layers of columnar and spongy tissue, shape of petiole on transverse section were noted.

Conclusion

Thus, the anatomical structure of the leaves of *Lonicera tatarica* and *Lonicera microphylla* was studied. It was determined that the leaves are light-type, with a dorsoventral structure in cross section, and petioles reinforced with areas of sclerenchyma. The mesophyll of the leaf blade is differentiated into columnar and spongy tissues. The stomata are small, few in number, anomocytic, and usually located on the lower side of the leaf. Round calcium oxalate crystals are present in the structure of the leaf blade and petiole.

Distinctive features of the leaves of both species are the shape of the epidermal cells, the number of layers of columnar and spongy tissue (*Lonicera microphylla* has more layers), the shape of the conducting bundles (broad and narrow-ovoid), the number of calcium oxalate crystals and the presence of simple trichomes on the underside of the leaf in *Lonicera microphylla*, in the absence of trichomes in the structure of *Lonicera tatarica*.

The data obtained confirm the xeromesophytic structure of the leaves and also show differences between plants for species identification based on vegetative organs.

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Author contribution

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Gavrilkova E.A**. — Investigation, Methodology, Plant material collection, Writing-review & editing; **Tleukenova S.U**. — Conceptualization, Data curation; **Ageev D.V**. — Plant anatomy; **Ramazanov A.K**. — Data curation, visualization.

Conflict of Interest

Authors declare no conflict of interest.

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Lonicera tatarica және *Lonicera microphylla* жапырақтарының салыстырмалы анатомиялық талдауы

Өсімдіктердің вегетативті мүшелерінің анатомиялық құрылымын зерттеу түрлердің биологиялық және экологиялық ерекшеліктерін бағалауға және таксондарды микроскопиялық деңгейде анықтауға мүмкіндік беретін құрылымдық ерекшеліктерді анықтауға мүмкіндік жасайды. Бұл әсіресе таксономиялық жақын түрлер үшін өте маңызды. Мақалада *Lonicera tatarica* және *Lonicera microphylla* жапырақтарының салыстырмалы анатомиялық зерттеу нәтижелері келтірілген. Жапырақ үлгілері 2024 жылдың жазында жиналды, Штраус-Флеминг ерітіндісінде тіркелді, беттік препараттар мен көлденең қималар қолмен жасалды. Зерттеу нәтижелері үшқаттар тұқымдасының екі түрі де бағаналы және кеуекті тәрізді ұлпаларға дифференциалданған мезофиллі бар дорзо-вентральды типтегі, жарық жапырақтарымен сипатталатынын анықтады. Жапырақ саңылаулары аз, аномоциттік типті, негізінен жапырақтың төменгі жағында локализацияланған. Екі түрде де кальций оксалатының ұсақ дөңгелек друзалары бар. Түрлер арасындағы айырмашылықтар жапырақ эпидермисінің жасушалары түрінде, бағаналы және кеуек тәрізді тіндердің қабаттарының саны, өткізгіш шоқтардың пішіні, кальций оксалатының друзаларының саны және қарапайым трихомалардың болуы.

Кілт сөздер: *Lonicera tatarica, Lonicera microphylla*, анатомиялық құрылымдар, жапырақ, қысқа шыбық, салыстырмалы зерттеу.

Е.А. Гаврилькова, С.У. Тлеукенова, Д.В. Агеев, А.К. Рамазанов

Сравнительный анатомический анализ листьев *Lonicera tatarica* и *Lonicera microphylla*

Изучение анатомического строения вегетативных органов растений позволяет оценить биологические и экологические особенности видов, а также выявить особенности строения, которые позволяют проводить идентификацию таксонов на микроскопическом уровне. Это особенно важно для таксономически близких видов. В статье представлены результаты сравнительного анатомического исследования листьев *Lonicera tatarica* и *Lonicera microphylla*. Образцы листьев были собраны в летний период 2024 г., зафиксированы в растворе Штрауса-Флеминга, поверхностные препараты и поперечные срезы выполнены вручную. Исследования показали, что оба вида жимолостей характеризуются световыми листьями, дорзо-вентрального типа с дифференцированным мезофиллом на столбчатую и губчатую ткани. Устьица немногочисленные, аномоцитного типа, локализованы преимущественно с нижней стороны листа. У обоих видов присутствуют мелкие округлые друзы оксалата кальция. Отличия между видами выявлены в форме клеток эпидермиса листа, количестве слоев столбчатой и губчатой ткани, форме проводящий пучков, количество друз оксалата кальция и присутствии простых трихом.

Ключевые слова: *Lonicera tatarica, Lonicera microphylla*, анатомические структуры, лист, черешок, сравнительное изучение.

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Scots pine (*Pinus sylvestris L.*) in natural and cultural populations of Central Kazakhstan (review)

The review summarizes recent findings on the morphological, anatomical, and biochemical characteristics of *Pinus sylvestris* L. in both natural and cultural established populations in Central Kazakhstan. Natural populations, occurring in the mountainous zones of the Kazakh Uplands, are characterized by ecological stability, climatic resilience, and high genetic diversity. In contrast, cultural plantations, established to mitigate desertification and stabilize soils, exhibit altered morphometric traits and a decrease in biodiversity due to monocultural practices. Comparative analysis of recent CIS and Kazakhstani studies reveals that environmental stressors, including technogenic pollution and soil degradation, significantly affect anatomical parameters of needles, radial growth, and phytochemical composition. The accumulation of heavy metals, decline in photosynthetic pigments, and variation in essential oil profiles reflect adaptive responses of *P. sylvestris* to anthropogenic impacts. The observed differences between natural and cultural populations underscore the need for region-specific forest management strategies, informed by anatomical and biochemical diagnostics. These findings support the development of improved selection and breeding programs tailored to Kazakhstan's diverse ecological zones.

Keywords: Pinus sylvestris L., Central Kazakhstan, natural and cultural populations, needle anatomy, morphological variability, biochemical adaptation, forest management, environmental stress.

Introduction

Pinus sylvestris L. (Scots pine) is a widely distributed coniferous species of significant ecological, silvicultural, and economic value across the Eurasian continent. Its natural range extends from Western Europe to Eastern Siberia, exhibiting remarkable adaptability to diverse climatic zones, including the semi-arid and strongly continental environments of Central Kazakhstan [1]. Within this region, *P. sylvestris* occurs in both natural and anthropogenically established populations, each fulfilling distinct ecological and land-use functions [2, 3].

Natural populations of Scots pine in Central Kazakhstan are predominantly located in mountainous and forest-steppe zones, particularly within the Karaganda and Ulytau regions. These relict forest communities are ecologically stable systems that contribute to biodiversity conservation, microclimate regulation, and soil stabilization. In contrast, cultural (planted) populations—mainly established in the middle of the XX century—are cultural afforestations intended to combat desertification, prevent soil erosion, and support regional timber production. These plantations are typically monocultures and experience different ecological pressures than their natural counterparts.

Despite the wide distribution of *P. sylvestris*, comparative studies focusing on the ecological and genetic characteristics of natural versus cultivated populations remain limited. In Kazakhstan and other CIS countries, various biological aspects of the species have been explored, including anatomical structure, genetic diversity, physiological stress responses, and the chemical composition of pine needles. However, integrated reviews assessing the adaptive capacities, ecological roles, and long-term sustainability of natural and cultural populations remain scarce [4, 5].

This review aims to consolidate and analyze existing scientific literature on *Pinus sylvestris* populations in Central Kazakhstan. It focuses on their distribution, morphological and anatomical traits, chemical composition, genetic structure, and practical applications, with particular attention to the distinctions between natural and cultivated populations. By evaluating their ecological functions and adaptive strategies, this review provides a scientific basis for developing sustainable forest management and conservation policies.

Ultimately, understanding and differentiating between natural and cultural populations is not only critical for biological and ecological research, but also essential for designing effective strategies for forest restoration, biodiversity conservation, and adaptive forest management in arid and semi-arid regions.

Experimental

Review method. This review was conducted through a structured analysis of scientific literature related to *Pinus sylvestris* (Scots pine) in Central Kazakhstan and adjacent Eurasian regions. Relevant publications were identified using academic databases such as Google Scholar, ScienceDirect, Scopus, and eLibrary.ru. Additional sources were gathered from national forestry research institutes and university repositories.

The selection criteria included: Studies published between 2000 and 2024; peer-reviewed articles, conference proceedings, and dissertations; research focusing on natural and/or cultural populations of *P. sylvestris*; publications providing data on morphological, anatomical, phytochemical, ecological characteristics; comparative or region-specific (Kazakhstan/CIS) studies.

Climatic conditions of Central Kazakhstan. Central Kazakhstan is characterized by a sharply continental and semi-arid climate. Average annual precipitation ranges between 200–350 mm, falling mostly during spring and early summer. The soil types are predominantly light chestnut and sandy soils, often prone to wind erosion and desertification. These harsh climatic and edaphic conditions significantly influence the growth, structure, and distribution of *P. sylvestris*, making it a relevant model species for studying adaptation and resilience in arid ecosystems.

Literature sources. The review synthesized data from over 30 scientific sources, including both Kazakhstani and CIS-based studies.

Key contributors from Kazakhstan include: Kopabaeva A. (2019) on dendrochronological and ecological analysis; Elkenova B.Z. et al. (2020) on pollution impact on needle structure; Krekova Yu. et al. (2023) on genetic diversity in northern populations [6–8].

From the wider CIS and international context, studies by: Lebedev A., Kuzmichev V.V. (2021) on biomass dynamics under climate change; Ermakov N.B. (2020) on pine forest classification; Kandziora-Ciupa M. (2016) on heavy metal accumulation and antioxidant response; were critically evaluated for comparative insights [9–11]. These sources provided a foundation for comparing natural and cultural populations in terms of: growth patterns; resistance to environmental stress; biodiversity support; chemical and genetic indicators of ecological fitness.

Results and Discussion

Literature review. Scots pine *(Pinus sylvestris L.)* is one of the most widely distributed and ecologically significant conifer species of the Northern Hemisphere, thriving across a wide range of climates and landscapes in Eurasia. Its natural populations in Central Kazakhstan are primarily found in mixed coniferous forests in mountainous and foothill regions. These populations have adapted to the harsh continental climate, marked by sharp seasonal temperature variations and low precipitation. Due to long-term ecological adaptation, their genetic structure is diverse. These forests are ecologically important for maintaining biodiversity and contributing to carbon sequestration. Natural and cultural populations in Central Kazakhstan. The introduction of *P.sylvestris* into forest management and afforestation programs in Central Kazakhstan began during in the middle of the XX century**.** Cultural plantations were established to combat desertification, stabilize sandy soils, and mitigate soil erosion. These cultural pine forests also serve as a source of timber and contribute to local economies [12]. However, monoculture plantations are increasingly criticized due to their potential to decrease soil fertility, reduce biodiversity, and disrupt native plant communities [13, 14]. Several studies have documented changes in soil properties—such as decreased nitrogen content and reduced microbial activity—under *P.sylvestris* plantations [15, 16]. Recent studies in the CIS have evaluated the physiological response, morphological changes, and biochemical adaptations of *P.sylvestris* under various environmental conditions. Research has shown that the species growth and needle structure are sensitive to drought, industrial emissions, and soil contamination.

Table 1

Data from recent studies on the species of *Pinus sylvestris* studied in CIS

Species	Geographical location	Key Findings	Recent research (authors, year)
Pinus sylvestris	Europe	Long-term changes in biomass due to climate shifts since 1940	Lebedev A., Kuzmichev V.V. (2021)
	Ai-Petri, Crimea	Natural reforestation and regeneration patterns in high-altitude forests	Saltykov A.N. (2023)

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Continuation of Table 1

Species	Geographical location	Key Findings	Recent research (authors, year)
Pinus sylvestris	Northern Eurasia	Classification of pine forests; ecological-geographical zonation	Ermakov N.B. (2020)
	Russia	Chemical composition in pine needles across age gradients	Yustina Potashkina (2024)
	Poland	Accumulation of heavy metals and increased anti-oxidant response in polluted areas	Kandziora-Ciupa M. (2016)
	Middle Ural, Russia	Morphological and biochemical shifts in trees growing on technogenic mine substrates	Chukina et al. (2025)
	Krasnoyarsk, Russia	Radial growth modified by climatic and pollution factors	Kladko et al. (2023)
	Siberia	Tree rings as geochemical indicators of past con-tamination	Mironova et al. (2020)
	Kostanay Region, Kazakhstan	Morphometric changes in needles due to technogenic pollution	Bragina et al. (2024)
	Karelia, Russia	Effectiveness of pine forest plantation methods on grassy clearings	Gavrilova et al. (2023)
	East Kazakhstan	Needle responses to asbestos tailing dumps; bio-chemical stress reactions	Chukina et al. (2024)

Table 1 presents key research findings from CIS and European studies on *P.sylvestris*, highlighting its responses to environmental stressors such as industrial pollution, soil degradation, and climatic fluctuations. Many of these studies emphasize the morphological, anatomical, and biochemical shifts observed under technogenic conditions, including changes in radial growth, heavy metal accumulation, and antioxidant activity.

Table 2

Data from recent studies on the species of *Pinus sylvestris* studied in Kazakhstan

Species	Geographical location	Key Findings	Recent research (authors, year)
Pinus sylvestris	The Small hills of Central Kazakhstan	Dendrochronological analysis of growth dynamics under climate change	Kopabaeva A. (2019)
	Semey region, East Kazakhstan	Decreased needle length and annual increment due to industrial emissions	Elkenova et al. (2020)
	North Kazakhstan	Genetic diversity of half-sib families and growth variability	Krekova et al. (2023)
	"Irtysh forest" re-serve, East Kazakh-stan	Study of ectomycorrhizal symbiosis with *Pinus sylvestris*	Nurlabi et al. (2023)
	Kostanay region	Morphometric and necrotic needle changes across polluted and clean sites	Bragina, Shvan (2024)
	Burabay, North Ka-zakhstan	Chemical variability in essential oils in pine needles under varying ecological conditions	Aidarkhanova et al. (2022)
	Kazakh uplands	Forest site conditions and their relation to reforesta-tion success	Makeeva et al. (2014)
	"Semey ormany" Natural reserve	Health condition of pine stands across forest size categories	Zalesov et al. (2015)
	Beskaragay, Bayanaul regions	Climatic effects on radial growth in forest ecosys-tems	Zhumadina et al. (2019)
	Northeast Kazakh-stan (ribbon pine forests)	Suppressed growth due to mass outbreaks of gypsy moth	Mapitov, Zhumadina (2015)
	Central Kazakhstan (several settlements)	Comparative anatomical assessment of pine needles from different populations	Tuleshova (2023)

A cross-comparison of the studies presented in Tables 1 and 2 highlights the differing research priorities between international/CIS-based studies and those conducted in Kazakhstan. While the former focus heavily on stress response mechanisms to pollution and climate variability, the latter prioritize morphological and anatomical changes under region-specific conditions. Notably, Kazakhstani studies contribute uniquely to understanding adaptation in semi-arid continental ecosystems, providing data that is underrepresented in broader Eurasian reviews.

Genetic and breeding research. In a pan-European context, *P.sylvestris* has been the subject of extensive breeding programs. Studies by Krakau et al. highlight how different European countries have advanced or halted breeding programs based on national priorities. Somatic embryogenesis and selection of elite genotypes are current focal areas of breeding research. In CIS countries, particularly Russia, extensive investigations into the genetic variability and selection potential of Scots pine populations have been carried out to improve forest productivity and resilience.

Comparative analysis of literature: CIS and International studies and Kazakhstan-based research. The literature presented in Table 1 and 2 highlights the diversity of scientific approaches and ecological contexts in which *P.sylvestris* populations have been studied. A comparative analysis reveals both commonalities and region-specific focuses that are essential for understanding the ecological plasticity and physiological responses of Scots pine across Eurasia.

Studies from CIS countries and Europe (Table 1) predominantly investigate the effects of technogenic pollution, climatic stressors, and forest management strategies on the growth and survival of *P.sylvestris.* For instance, Kandziora-Ciupa (2016) reported the accumulation of heavy metals and the activation of antioxidant responses in pine needles collected from polluted sites in Poland. Similarly, Chukina et al. (2025), and Potashkina (2024) documented significant anatomical, physiological, and biochemical changes in *P.sylvestris* growing under technogenic and mining conditions in Russia, emphasizing the species stress response mechanisms [17–23].

In contrast, the studies conducted in Kazakhstan (Table 2) largely emphasize the species adaptation to harsh continental climates, with a specific focus on morphological and anatomical traits (Tuleshova et al., 2023; Bragina, Shvan et al., 2024), radial growth dynamics under varying climatic and ecological conditions (Zhumadina et al., 2019; Mapitov et al., 2015), and phytochemical composition of pine needles (Aidarhanova et al., 2022). These studies reflect a strong regional interest in ecological monitoring, afforestation strategies, and the assessment of forest health in semi-arid environments [24–28]. Methodologically, international and Russian studies tend to employ a broader spectrum of analytical tools, including dendrochronological series, geochemical monitoring, somatic embryogenesis (Krakau et al., 2013), and advanced spectroscopic techniques [29]. In Kazakhstan, while modern chromatographic and microscopic techniques are applied, many investigations remain practice-oriented, focusing on local ecological indicators and applied forestry. In terms of practical implications, the research conducted in Europe and the CIS underscores the importance of genetic improvement, forest productivity, and resilience under environmental stressors. Conversely, Kazakhstan-based studies contribute valuable insights into the viability of *P.sylvestris* in afforestation projects, particularly under challenging environmental and climatic constraints of Central Asia [30]

In conclusion, while both datasets demonstrate the ecological versatility of *P.sylvestris*, the Kazakhstan studies provide a unique contribution to understanding the species adaptation strategies in arid-steppe and semi-arid conditions. Integrating findings from these different geographical contexts enhances our understanding of the species ecological amplitude and supports the development of region-specific conservation and forest management strategies.

Conclusions

Overall, *Pinus sylvestris* is not only a key species in natural ecosystems but also a vital component of afforestation and land stabilization projects in Central Asia. While natural populations exhibit high adaptive potential and ecological value, cultural plantations pose challenges related to biodiversity and soil health. Continued interdisciplinary research combining anatomical, biochemical, dendrochronological, and ecological approaches is essential for sustainable pine forest management and breeding programs in Kazakhstan and beyond.

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Author Contributions

Tuleshova K.A. – Data curation, Investigation, Conceptualization, Methodology. **Kali A.K.** – Supervision, Writing draft, Editing. **Silantyeva M.M.** – Formal analysis, Project administration.

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Орталық Қазақстандағы табиғи және дақылды популяциялардағы кәдімгі қарағай (Pinus sylvestris L.) (шолу)

Бұл шолу жұмыста Орталық Қазақстандағы *Pinus sylvestris* L. (кәдімгі қарағай) табиғи және дақылды популяцияларының морфологиялық, анатомиялық және биохимиялық ерекшеліктеріне қатысты соңғы зерттеу нәтижелері қарастырылған. Табиғи популяциялар негізінен Қазақтың ұсақ шоқыларының таулы аймақтарында орналасқан және экологиялық тұрақтылығымен, климатқа бейімділігімен, жоғары генетикалық әртүрлілігімен ерекшеленеді. Ал шөлейттену мен топырақ эрозиясымен күресу мақсатында жасанды түрде отырғызылған дақылды популяциялар моноқатарлы құрылым салдарынан морфометриялық өзгерістерге ұшырап, биоалуантүрліліктің төмендеуіне себеп болуда. ТМД елдері мен Қазақстанда жүргізілген салыстырмалы зерттеулер көрсеткендей, техногендік ластану мен топырақтың деградациясы қылқанның анатомиялық құрылымына, радиалды өсуіне және фитохимиялық құрамына айтарлықтай әсер етеді. Ауыр металдардың жиналуы, фотосинтездік пигменттердің азаюы, сондай-ақ эфир майлары құрамындағы өзгерістер ағаштардың бейімделу реакциясын сипаттайды. Табиғи және дақылды популяциялар арасындағы бұл айырмашылықтар орман шаруашылығын басқаруда, селекциялық және қорғау стратегияларын жетілдіруде маңызды екенін көрсетеді.

Кілт сөздер: Pinus sylvestris L., Орталық Қазақстан, табиғи және дақылды популяциялар, қылқандар анатомиясы, морфологиялық өзгергіштік, биохимиялық бейімделу, орман шаруашылығы, экологиялық күйзеліс.

К.А. Тулешова, А.К. Кали, М.М. Силантьева

Обыкновенная сосна (Pinus sylvestris L.) в природных и культурных популяциях Центрального Казахстана (обзор)

В обзоре обобщены современные исследования морфологических, анатомических и биохимических особенностей *Pinus sylvestris* L. (сосны обыкновенной) в природных и культурных популяциях Центрального Казахстана. Природные популяции, произрастающие в горных районах Казахского мелкосопочника, характеризуются высоким генетическим разнообразием, устойчивостью к континентальному климату и стабильностью экосистем. В то же время культурные насаждения, созданные с целью борьбы с опустыниванием и эрозией почв, демонстрируют морфометрические изменения, снижение биоразнообразия и трансформацию почвенных свойств вследствие монокультурного подхода. Сравнительный анализ исследований, проведённых в странах СНГ и Казахстане, показывает значительное влияние техногенной нагрузки и деградации почвы на анатомические параметры хвои, радиальный прирост и фитохимический состав. Накопление тяжёлых металлов, снижение содержания фотосинтетических пигментов и изменение эфирномасличного профиля являются реакцией на экологический

стресс. Выявленные различия между природными и культурными популяциями подчёркивают необходимость адаптации лесохозяйственных стратегий и совершенствования селекционных программ с учётом региональных экологических условий.

Ключевые слова: Pinus sylvestris L., Центральный Казахстан, природные популяции, культурные лесонасаждения, анатомия хвои, морфологическая изменчивость, биохимическая адаптация, лесное хозяйство, экологический стресс.

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