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Assessment of the effectiveness of ITS markers in DNA barcoding of *Betula* species in the Kazakh Altai

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Abstract. This study investigates the effectiveness of ITS (Internal Transcribed Spacer) DNA barcoding markers for identifying and differentiating eight species of *Betula* within the Kazakh Altai flora: *Betula glandulosa, B. pendula, B. pubescens, B. rotundifolia, B. rezniczenkoana, B. tianschanica, B. microphylla, and B. kirghisorum.* By extracting and sequencing DNA from herbarium specimens, the study focuses on assessing the genetic diversity and resolving phylogenetic relationships among these *Betula* species, many of which have close morphological similarities that can complicate traditional taxonomy. The results reveal that ITS markers provide clear genetic differentiation between these species, highlighting the effectiveness of ITS in distinguishing even closely related species within the Betula genus. Analysis using the Neighbor-Joining method showed two primary clusters, aligning well with known phylogenetic sections, *Apterocaryon* and *Betula*, which are established classifications within the genus. Furthermore, the study offers the first ITS sequence data for two species, *B. rezniczenkoana* and *B. kirghisorum,* contributing new genetic information to the NCBI database. These findings are crucial for ongoing conservation efforts, given the ecological and environmental pressures facing native Betula species in the Kazakh Altai. By providing reliable molecular markers, this research supports future studies on species distribution, genetic diversity, and conservation planning within this biodiverse and ecologically sensitive region.

Keywords: *Betula L.* species, ITS markers, DNA barcoding, evolutionary relationships, genetic variability

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Introduction

The genus *Betula* is an important component of biodiversity, and its study is crucial in the context of conservation and sustainable use of natural resources. In the face of anthropogenic impacts and global environmental changes, including climate change, understanding the genetic diversity and phylogenetic relationships within the Betulaceae family is essential for biodiversity conservation efforts and evolutionary research.

The genus *Betula* includes both trees and shrubs found in a wide range of habitats in the boreal and temperate zones of the Northern Hemisphere [1]. In the Kazakh Altai, Saur-Manrak, and Zaysan Depression, 15 species and 1 subspecies of the genus *Betula L.* have been identified. The Kazakh Altai is a unique and floristically diverse region, being one of the richest floristic areas in Kazakhstan. Over 2,500 species of higher plants grow here, representing almost 50% of the total flora of the Republic of Kazakhstan [2]. The species diversity is due to the region's unique climate, which has led to the formation of various ecological niches with meadows, wetlands, deserts, and semi-deserts.

In the ecosystem of the Kazakh Altai, the genus *Betula* plays a significant role, exerting an important influence on the natural environment and human activities. It contributes to landscape formation, forest resource management, biomass production, and horticulture, and also possesses high pharmacological potential [3-5]. Many birch species, being fast-growing and adaptable to various environmental conditions, can participate in the restoration of forest ecosystems in cleared or burned areas [6].

The taxonomy of *Betula* remains a subject of scientific discussion, with many issues unresolved due to the high variability of traits and hybridization. Studies by Touchette et al. [7] and Tsuda et al. [8] have examined the genetic structure and hybridization within the genus *Betula*, highlighting the complexity of *Betula* species across Eurasia.

The first author to provide a comprehensive review of the genus was Regel [9], who divided the genus into the subgenera *Betulaster* and *Eubetula.* The subgenus *Betulaster* contained only one section – *Acuminatae*. The subgenus *Eubetula* included six sections: *Costatae, Lentae, Nanae, Albae, Fruticosae*, and *Dahuricae.* Recent phylogenomic analysis of the plastome has offered a new perspective on the phylogeny and evolution of *Betulaceae*, highlighting the monophyletic relationships between genera such as *Betula* and *Alnus* [10].

The contemporary classification presented by Ashburner and McAllister is based on the analysis of phylogenetic relationships within the genus *Betula* L., morphological traits, habitat, and species ploidy. According to this classification, the genus Betula is divided into four subgenera and eight sections. These subgenera are: *Acuminata* (section Acuminatae), *Aspera* (section Asperae и Lentae), *Betula* (section Apterocaryon, Betula, Costatae, and Dahuricae), and *Nipponobetula* (section Nipponobetula). Additionally, the section Asperae is further divided into two subgroups: the section *Asperae* and *Chinenses.*

The chromosome number in *Betula* L. is n = 14. *Betula* species form a polyploid series with chromosome counts of 2n = 28, 56, 70, 84, and 112 [11]. Hybridization and introgression are common in *B. pendula* (2n = 28) and *B. pubescens* (2n = 56) due to overlapping ranges of the species [12]. Ashburner and McAllister suggest that *B. microphylla* (2n = 56) and *B. tianschanica*

(2n = 56) are hybrids of the parent species *B. fruticosa* (2n = 56), *B. pubescens, B. utilis* (2n = 56), and *B. pendula*.

Polyploidy occurs in species of different subgenera, indicating several independent polyploidizations within the genus and serving as an important criterion for distinguishing some morphologically similar species, such as the diploid B. pendula (2n = 2x = 28) and the tetraploid *B. pubescens* (2n = 4x = 56).

Traditional methods of species identification in birches often fall short due to their high phenotypic plasticity and frequent hybridizations, which blur the boundaries between different taxa [13]. Therefore, one of the methods for studying biodiversity and genetic identification is DNA barcoding [14]. Recently, this method has been considered one of the most useful and objective "tools" for species identification based on the diversity of marker gene sequences from nuclear and plastid genomes. Additionally, DNA barcoding allows for the establishment of evolutionary relationships between different *Betula* L. species and helps understand genetic diversity and evolutionary patterns within the genus.

A significant number of studies highlight the high effectiveness of using nuclear genome markers – ribosomal internal transcribed spacers (ITS) (Figure 1). These are among the most thoroughly characterized and rapidly evolving genes, and their resolution is quite high when used together, making them a powerful tool for the identification and classification of birches [15]. Analysis of the chloroplast genome provides insights into maternal evolutionary relationships between species, while biparentally inherited nuclear DNA offers independent data for inferring evolutionary relationships [16-18].

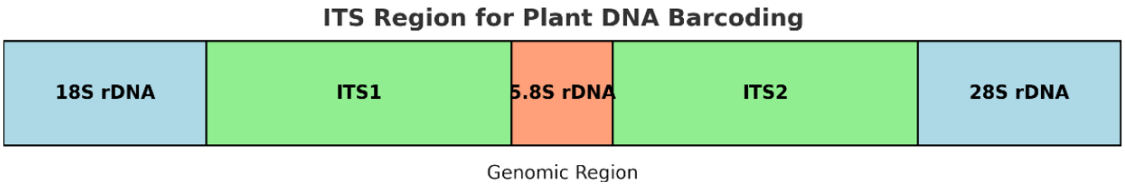


Figure 1. Diagram of the ITS Region

The effectiveness of markers for DNA barcoding in plants is determined by both the ease of amplification and the ability to distinguish species. The ITS marker shows a high amplification rate of ~90% and a strong capability for species differentiation [18]. However, species identification of *Betula* L. using barcoding faces several challenges, such as genetic variability, adaptation to environmental conditions, limited availability of reference sequences, and changing regional ecology. Additionally, the genus *Betula* L. is considered one of the most complex among all circumpolar genera [17].

These issues in the systematics of the genus underscore the relevance of the research presented in this study. This research aimed to determine the genetic diversity and establish relationships between different *Betula* L. species found in the Kazakh Altai.

Materials and research methods

The study focused on herbarium specimens of 8 *Betula* species: *Betula*: *B. glandulosa, B. pendula, B. pubescens, B. rotundifolia, B. rezniczenkoana, B. tianschanica*, and *B. microphylla, B. kirghisorum* collected from the Kazakh Altai region (Table 1). Botanical identification of the species was conducted by the staff of the RSE "Altai Botanical Garden". Taxonomic information for the samples was obtained from herbarium labels (Figure 1).

Table 1

DNA Collection of Endemic and Rare Plant Species from the Kazakh Altai

Species	Coordinates	Elevation	Geographical and Administrative Location
Betula glandulosa	50.32694 83.54556	1870	Sarymsakty Ridge, Burkhat Pass
Betula pendula	50.31933 83.89556	1180	Northwest Slope of Ivanovsky Ridge
Betula pubescens	50.32028 84.19556	1935	Koksinsky Ridge Altai Botanical Garden
Betula rotundifolia	50.1934 83.3246	774	Altai Botanical Garden
Betula rezniczenkoana	50.1934 83.3246	774	Altai Botanical Garden
Betula tianschanica	50.1934 83.3246	774	Northwest Slope of
Betula microphylla	49.18972 85.51944	820	Mount Bukhtarma
Betula kirghisorum	51.101 73.3356	101	Qaragandy region

Samples were homogenized using an automatic homogenizer TissueLyser LT (Qiagen, Germany). Genomic DNA was extracted from herbarium specimens using a modified acidic CTAB extraction buffer (2% CTAB, 2 M NaCl, 10 mM Na3EDTA, 100 mM HEPES, pH 5.3) with RNase A treatment. DNA was dissolved in 1×TE buffer (1 mM EDTA, 10 mM Tris-HCl, pH 8.0). DNA quality was assessed spectrophotometrically using a Nanodrop (Thermo Fisher Scientific Inc., Waltham, MA, USA) and by running a 1% agarose gel stained with ethidium bromide at 90 V for 20 minutes.

Amplification was conducted in a final volume of 25 µL. The amplification mixture included 10 ng of DNA, PCR buffer (2 mM MgSO4; 10 mM KCl; 10 mM (NH4)2SO4; 20 mM Tris-HCl, pH 8.8), 5 pmol of primers, 200 µM dNTPs, and Phire Hot Start II DNA Polymerase (Thermo Fisher Scientific). PCR amplification of DNA was performed using ITS primers on a ThermalCycler (Thermo Fisher Scientific). Primer sequences are provided in Table 2.

Initial denaturation was performed at 98°C for 3 minutes, followed by 15 seconds at 98°C. The subsequent 35 amplification cycles were conducted with annealing temperatures ranging from 55°C to 58°C for 30 seconds and extension at 72°C for 60 seconds. A final extension was carried out at 72°C for 2 minutes.

Table 2

Primer Sequences for DNA Barcoding

№	Primer	Sequence 5' - 3'	GC%	Tm °C	Amplicons
1	ITS4	TCCTCCGCTTATTGATATGC	45	62	400-680bp
2	ITS5	GGAAGTAAAAGTCGTAACAAG	38	58	

Amplification results were checked using a 1.2% agarose gel stained with ethidium bromide (Figure 1). The sizes of the amplified DNA fragments were determined by comparing them to a marker (Thermo Scientific GeneRuler DNA Ladder Mix 100-10,000 bp). Fragment lengths were analyzed using the iBright 1500 Imaging System for gel documentation.

Figure 1. Herbarium samples of various *Betula* species

PCR products were purified using the ExoSap-IT PCR Product Cleanup Kit (Applied Biosystems, Inc., USA) and visualized on a 1.2% agarose gel. Sequencing was performed with ITS-specific

primers using the ABI3700 capillary sequencer (Applied Biosystems Thermo Fisher Scientific) and Sanger sequencing (BigDye® Terminator chemistry). Visualization and analysis of the data were conducted using SeqMan 6.1 software.

Sample identification was based on the analysis of the primary nucleotide sequences compared against the GenBank database (http://www.ncbi.nlm.nih.gov) using multiple sequence alignment in MEGA 11 [19]. The evolutionary divergence between nucleotide sequences of the plant species studied was calculated based on the ITS sequences from all eight populations of the Betula L. genus using MEGA 11 software.

Results

In our study, DNA was extracted from herbarium samples of *Betula* collected at the Altai Botanical Garden. The ITS marker used for barcoding demonstrated high reproducibility of results. It is noteworthy that the success of obtaining DNA sequences varied among taxa. Generally, the success of DNA extraction and subsequent amplification was dependent on the quality of the herbarium material and the chosen plant part. The sizes of the amplified regions varied according to the primers used and matched the expected values: the ITS2 fragment sizes ranged from 423 to 670 base pairs (Figure 2).

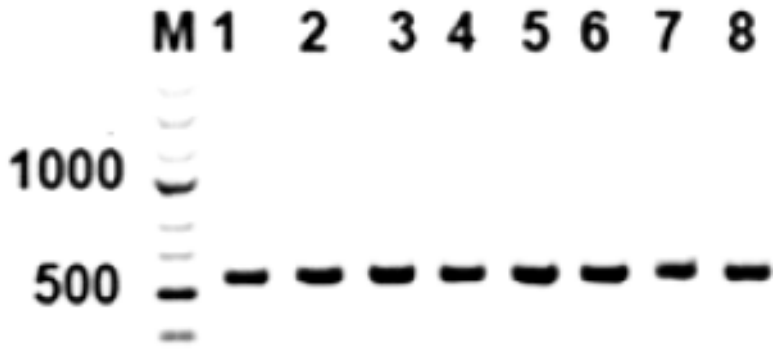


Figure 2. Results of DNA Amplification for Herbarium Samples of the Betula Genus Using ITS Region Primers

The sequencing results included a chromatogram for each sample and a file with its interpretation. The ITS nucleotide sequences for the *Betula* genus ranged from 402 to 638 base pairs. Figure 3 displays the alignment of nucleotide sequences for *Betula* L. samples, with differences in sequences highlighted by boxes, indicating the presence of polymorphic regions. For instance, variations in *Betula glandulosa* and *Betula microphylla* suggest that they belong to the same section, *Apterocaryon.*

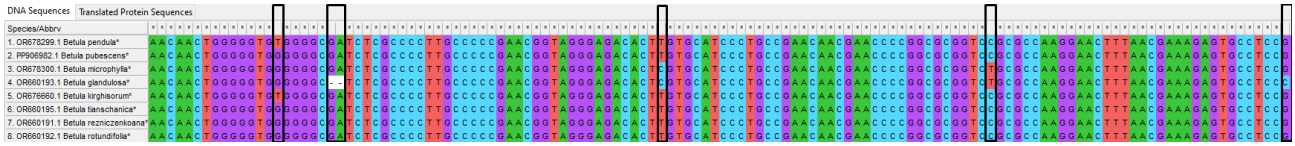


Figure 3. Fragment of the multiple alignment of ITS sequences for *Betula L.* samples using MEGA X software

The variability in the length and nucleotide composition of the ITS marker reflects the genetic diversity within the *Betula* genus. These results are consistent with similar studies and confirm the complex evolutionary history of the genus [20-23].

The average nucleotide composition for the ITS marker is as follows: T = 18.9%, C = 30.9%, A = 20.2%, and G = 29.8%. The highest AT content (40.6%) and the lowest GC content (59.5%) were observed in the *B. kirghisorum* population, while the lowest AT content (38.4%) and the highest GC content (61.6%) were found in the *B. tianschanica* population (Table 4). Nucleotide frequencies calculated according to the Tamura-Nei model [19] for the ITS marker are: 19.87% (A), 19.14% (T/U), 31.10% (C), and 29.90% (G).

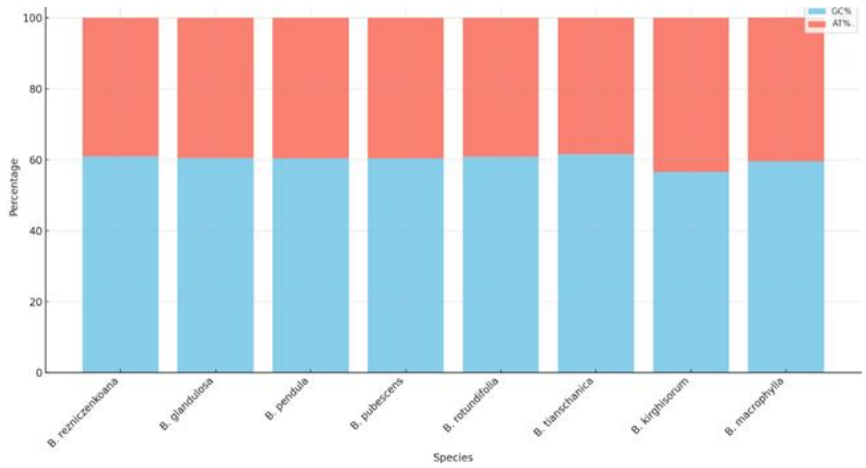


Figure 4. AT% and GC% by Betula sp for ITS

The analysis of nucleotide substitutions reveals a predominance of transition substitutions over transversions. This suggests their relative conservativeness and significance for evolutionary studies within the genus *Betula*. Transitions (substitutions between purines or pyrimidines) are less disruptive to the structural integrity of DNA than transversions (substitutions between a purine and a pyrimidine).

Table 4

Evaluation of Maximum Likelihood Estimates of Nucleotide Substitution Patterns for ITS

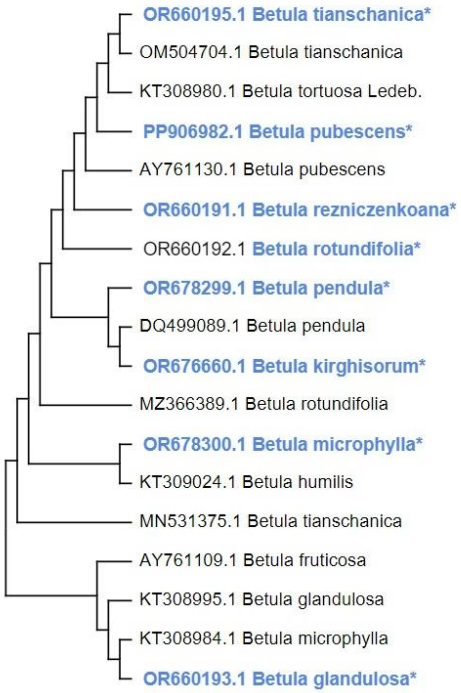
	A	T	C	G
A	-	4,764	7,56	12,07
T	5,00	-	18,99	7,29
C	5,00	11,67	-	7,29
G	8,28	4,64	7,56	-

Note: Frequencies of different transition substitutions are shown in bold, while frequencies of transversion substitutions are shown in italics

Transition substitutions between purines (A↔G) and pyrimidines (C↔T) are detailed in Table 4. The frequency of C↔T substitutions is 18.99%, indicating a high probability of this type of substitution. In contrast, transversion substitutions between purines and pyrimidines show lower frequencies, with A↔C and A↔T substitutions at 5, and G↔C and G↔T substitutions varying at 7.56 and 7.29, respectively.

The use of DNA barcoding successfully identified species in all cases, with consensus marker sequences aligning 98-100% with known sequences. Consequently, we have deposited eight sequences in GenBank: OR660193 *(B. glandulosa),* OR660156 *(B. pendula),* OR821848 *(B. pubescens)*, OR660192 *(B. rotundifolia),* OR660191 *(B. rezniczenkoana),* OR660195 *(Betula tianschanica),* OR660176 *(B. microphylla),* and OR660092 *(B. kirghisorum).* This ensures open access to the data and supports the transparency of the research.

The importance of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA for elucidating the informative nature of ITS sequences in assessing the phylogenetic relationships within the Betula genus was demonstrated by Chen et al. [16]. A phylogenetic tree was constructed using the Neighbor-Joining (NJ) method, with reference sequences downloaded from the NCBI database for the Betulaceae family. This tree revealed diversity in the clustering of *Betula* species. Additionally, *B. tortuosa, B. himilis,* and *B. fruticosa* from the Altai region of Kazakhstan were included in the analysis (sequence data for these species were obtained from GenBank).



Note: indicates sequences downloaded from GenBank

Figure 4. Phylogenetic tree of Kazakhstani Betula species constructed using the Neighbor-Joining method based on ITS sequencing results

Discussion

According to the classification by Ashburner K and McAllister, all analyzed samples belong to the subgenus *Betula* [13, 24]. The use of the ITS marker allowed the construction of a phylogenetic tree and the identification of 2 clades. The species B. tianschanica, B. pubescens, B. pendula, and B. glandulosa were grouped in one clade along with their reference sequences.

The first clade is characterized by the fact that *Betula* pubescens Ehrh. often forms hybrids with *B. pendula* Roth, *B. rezniczenkoana* (Litv.) Schischk., and *B. microphylla* Bunge, which complicates their diagnosis [2]. *B. rezniczenkoana* (Litv.) Schischk. is a hybrid resulting from the cross between *B. pendula* Roth and *B. microphylla* Bunge [25]. Other sources also indicate hybridization between *B. pendula* Roth and *B. tianschanica* Rupr [26].

Betula rotundifolia grouped into a clade with its reference sequence from the NCBI database, but not in immediate proximity, which may be related to difficulties in species identification and hybridization with *B. pendula* Roth (B. × pseudomiddendorffii V.Vassil.). For the *Betula* kirghisorum sample, as with *B. rezniczenkoana*, there are no reference sequences in the NCBI database, making these species less studied compared to the other analyzed samples.

In the second clade of the *Apterocaryon* section, *B. glandulosa* was placed close to its reference sequence. Interestingly, *B. microphylla* was included in the*Betula* section, although it is classified as part of the *Apterocaryon* section. This discrepancy may be due to the high polymorphism of *B. microphylla* [27]. Its significant genetic plasticity is attributed to the diverse habitat conditions and is reflected in a wide range of morphological variability used in birch diagnoses. When growing alongside other birch species (*B. pendula)*, it undergoes hybridization, producing various intermediate forms. It is also notable that *B. rotundifolia* and *B. microphylla* each had only one sequence in the international NCBI database, which limited the comparative analysis possibilities.

Nevertheless, our results align with previous studies by Järvinen et al. [17] and Fazekas et al. [28] and confirm that DNA barcoding using the ITS marker can successfully identify the *Betula* genus but lacks sufficient resolution for species differentiation [24]. The absence of reference sequences in the GenBank database for some species, such as *B. rezniczenkoana* and *B. kirghisorum,* highlights the need for further research and database expansion to improve the accuracy of phylogenetic analyses.

Our findings emphasize the importance of exploring and utilizing additional DNA regions as supplementary barcoding markers to resolve species-level identification in *Betula*. Future research should focus on investigating other potential barcoding regions and their applicability for differentiating *Betula* species and related taxa.

Conclusion

The results obtained expand our understanding of the genetic architecture and evolutionary relationships within the *Betula* L. species. Future integration of multi-marker approaches and enhanced sampling efforts will refine classification and provide a deeper understanding of the evolutionary dynamics of this taxonomic group. Such efforts are crucial for developing effective conservation strategies and sustainable management of *Betula* species in the face of ecological challenges.

Author Contributions

A.T. and **S.M. –** design of the study, collection, analysis, and interpretation of the results. **O.K.** – supervisor of the study, critically revising its content.

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Conflicts of Interest

The authors declare no conflicts of interest.

Compliance with ethical standards

This article does not contain a description of studies performed by the authors involving people or using animals as objects.

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Қазақстан Алтайындағы *Betula* түрлерінің ДНҚ штрих-кодтауында ITS маркерлерінің тиімділігін бағалау

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Аңдатпа. Бұл зерттеуде Қазақстан Алтайының флорасында өсетін сегіз қайың ағаш түрін (*Betula* glandulosa, *B. pendula, B. pubescens, B. rotundifolia, B. rezniczenkoana, B. tianschanica, B. microphylla* және *B. kirghisorum*) идентификациялау мен дифференциациялау үшін ITS (ішкі транскрибтелетін ДНҚ спайсері) штрих-код маркерін қолданудың тиімділігі зерттелген. ДНҚ-ны бөлу және гербарий үлгілерін секвенирлеу арқылы жүргізілген зерттеу арқылы генетикалық әртүрлілікті бағалауға және осы түрлер арасындағы филогенетикалық байланысты нақтылауға бағытталған, олардың көпшілігінің морфологиялық белгілері ұқсас болып келеді, бұл дәстүрлі таксономиялық жіктелуге қиындықтар туғызуы мүмкін. Нәтижелер көрсеткендей, ITS маркерлері зерттелген түрлер арасында анық генетикалық дифференциацияны қамтамасыз етеді, тіпті Betula туысының жақын туыстас түрлерін ажыратуда олардың тиімділігін растайды. Қосылған көрші әдісі (NJ әдісі) екі негізгі кластердің бар екенін анықтады, бұл Apterocaryon және *Betula* филогенетикалық топтары – туыстағы белгілі классификацияларға сәйкес келеді. Зерттеу сондай-ақ *B. rezniczenkoana* және *B. kirghisorum* түрлерінің ITS тізбегінің мәліметтерін алғаш рет ұсынады, бұл NCBI дерекқорында жаңа генетикалық ақпарат қосуға мүмкіндік береді. Алынған нәтижелер экологиялық мәселелерді ескере отырып, Қазақстан Алтайындағы жергілікті қайың ағашы түрлерінің биоәртүрлілігін сақтау үшін маңызды болып табылады. Сенімді молекулярлық маркерлерді ұсына отырып, бұл зерттеу түрлердің таралуын, генетикалық әртүрлілікті зерттеу және биологиялық тұрғыдан алуан түрлі әрі экологиялық тұрғыдан сезімтал аймақта сақтау шараларын жоспарлау бойынша жұмыстарды қолдайды.

Түйін сөздер: Қайың түрлері (*Betula* L.), ITS маркерлері, ДНҚ штрих-кодтауы, эволюциялық байланыстар, генетикалық өзгергіштік

Оценка эффективности ITS-маркеров в ДНК-штрихкодировании видов *Betula* в Казахском Алтае

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Аннотация. В данном исследовании изучается эффективность использования маркера штрих-кода ITS (внутренний транскрибируемый спейсер ДНК) для идентификации и дифференциации восьми видов березы, произрастающих во флоре Казахского Алтая: *Betula glandulosa, B. pendula, B. pubescens, B. rotundifolia, B. rezniczenkoana, B. tianschanica, B. microphylla* и *B. kirghisorum*. Путем выделения ДНК и секвенирования гербарных образцов исследование направлено на оценку генетического разнообразия и уточнение филогенетических связей между этими видами, многие из которых имеют сходные морфологические признаки, что может затруднить их традиционную

таксономическую классификацию. Результаты показали, что маркеры ITS обеспечивают четкую генетическую дифференциацию между изучаемыми видами, подтверждая их эффективность в различении даже близкородственных видов рода *Betula*. Анализ методом объединения соседей (NJ method) выявил наличие двух основных кластеров, которые согласуются с известными филогенетическими группами Apterocaryon и *Betula* – установленными классификациями внутри рода. Кроме того, в исследовании впервые представлены данные последовательности ITS для видов *B. rezniczenkoana* и *B. kirghisorum,* что добавляет новую генетическую информацию в базу данных NCBI. Полученные результаты имеют важное значение для сохранения биоразнообразия, учитывая экологические проблемы, с которыми сталкиваются местные виды берез Казахского Алтая. Предоставляя надежные молекулярные маркеры, это исследование поддерживает дальнейшие работы по изучению распространения видов, генетического разнообразия и планирования мер по сохранению в этом биологически разнообразном и экологически чувствительном регионе.

Ключевые слова: виды березы (*Betula* L.), ITS-маркеры, штрих-кодирование ДНК, эволюционные взаимоотношения, генетическая изменчивость

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Induced RNA interference and its impact on potato virus amplification in plants

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Abstract. Viral diseases in potato crops, especially Potato virus Y (PVY), Potato leafroll virus (PLRV), Potato virus M (PVM), and Potato spindle tuber viroid (PSTVd), are particularly difficult to control and represent an important problem for worldwide agriculture. In this study, we assessed the antiviral potency of RNA interference (RNAi) and CRISPR/Cas13 technologies in lowering viral titers and preventing pathogenesis.

Potato plants were inoculated with individual and combined viral pathogens and subsequently treated with constructs containing gRNA, sense, and antisense sequences used for both RNAi and Cas13-mediated degradation of the viral RNA. Quantitative PCR (qPCR) was utilized to measure RNA levels, and disease progression was observed for three weeks. Expression of Cas13 was confirmed by fluorescence microscopy and Western blot. Results of the RNAi constructs lowered levels of viral RNA up to 85% for PVY and 78% for PLRV. CRISPR/Cas13 constructs yielded even greater suppression rates (≥90% in some treatments) with marked symptom alleviation. Synergistic effects of constructs targeting multiple targets were observed with the greatest decreases in viral loads and disease severity. The differences were statistically significant ($p < 0.05$) between treated and control plants. This research demonstrates that RNAi and CRISPR/Cas13 can enhance potato resistance to viral infections. Providing a scalable, transgene-free approach to disease control, these methods contribute to sustainable agriculture and global food security.

Keywords: potato virus, RNA interference, CRISPR/Cas13, gene editing, virus suppression, molecular tools, sustainable agriculture, transgene-free technology

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Introduction

Potatoes *(Solanum tuberosum)* are a key staple crop worldwide and provide macronutrients and micronutrients for billions of people. The production of potatoes is threatened by a wide range of viral pathogens, such as Potato leafroll virus (PLRV), Potato virus Y (PVY), Potato virus M (PVM), and the viroid - Potato spindle tuber viroid (PSTVd). These infectious agents not only reduce tuber yield but negatively affect processed harvest tubers, making it critically important to protect potato plants from the negative impact of these relevant phytopathogens. This results in significant financial losses for farmers and agribusinesses across the globe. [1-10].

The management of viral diseases in potato crops remains a significant challenge due to the absence of effective chemical treatments and the rapid evolution of viral strains. Traditional approaches, such as breeding for resistance and vector control, are often labor-intensive and insufficient to counter the fast-paced emergence of new viral variants. Consequently, there is an urgent need for innovative strategies to protect potato crops from viral infections [11-14].

RNA interference (RNAi) and CRISPR/Cas systems have emerged as powerful tools for plant disease management. RNAi exploits the plant's natural defense mechanisms to silence specific viral genes, whereas CRISPR/Cas systems enable precise and programmable targeting of viral genomes for degradation. Among these, the Cas13 family of RNA-targeting enzymes has shown remarkable promise in combating RNA viruses due to its specificity and efficiency [15-17].

We investigated the effectiveness of RNA interference (RNAi) and CRISPR/Cas13 technologies on inhibiting the replication of key potato viral pathogens. Multiple studies have shown that both Cas13 as well as RNA constructs that target the gRNA (RNAi) are effective in reducing the severity of viral infections in potato plants [18-21]. We examined their capacity to reduce viral loads and symptoms in potato plants via probe- and gRNA-directed Cas13 constructs and sense/antisense RNAi constructs. This study on the use of such technologies for the management of viral infections has the potential to develop new and transgene-free, sustainable solutions for the protection of potato crops.

Here, we provide an overview of the design, implementation, and utility of these advanced molecular tools. We evaluate their potential benefits, limitations, and implications for enhancing global food security as agricultural challenges intensify.

Materials and research methods

Plant Materials and Experimental Design

The study utilized 51 economically significant potato cultivars (Solanum tuberosum), selected for their high agronomic traits and resistance potential. Virus-free plants were produced from the apical meristems of sprouted potato tubers (3-5 cm). All sprouts were thoroughly surface-sterilized before excision to ensure they were free from contamination. Details of the initial cleaning procedures, including detergent solutions containing Tween-20, and treatment with sterilizing agents such as ethanol, mercuric chloride, and hydrogen peroxide (Table 1), are provided. After sterilization, explants were washed 3-5 times with autoclaved distilled water to remove residual sterilizing agents.

Table 1

Variants of explant sterilization

No. Sterilization Option	Sterilizing Agent	Sterilization Time
1	70% Ethanol	20 s
		30 s
		50 s
2	0.1% Mercuric Chloride	1 minute
		2 minutes
		3 minutes
3	10% Hydrogen Peroxide	2 minutes
		5 minutes
		8 minutes

Sterilized plant explants were placed on Murashige and Skoog (MS) medium supplemented with sucrose, agar, and necessary vitamins. The pH prior to autoclaving was buffered to 5.7. Seven variations of growth media with different concentrations of plant growth regulators, including auxins, gibberellins, and cytokinins, were tested to optimize shoot regeneration (Table 2). Cultivation conditions were set to 25 ± 2°C under a 16-hour photoperiod with 8 hours of darkness using a climate chamber (Binder KBWF 240, Tuttlingen, Germany).

Table 2

Variants of growth media supplemented with phytohormones

Variants of MS Media	Indoleacetic Acid (IAA)	Indole-3-Butyric Acid (IBA)	Gibberellic Acid (GA)	Kinetin	6-Benzylaminopurine (6-BAP)
MC1	0.1	-	0.3	-	-
MC2	-	0.1	0.3	-	-
MC3	0.05	0.05	-	-	-
MC4	-	-	0.3	-	1
MC5	-	-	0.3	1	-
MC6	0.1	-	-	-	1
MC7	-	0.1	-	-	1

RNA Extraction and Pathogen Detection

Total RNA was extracted from 250 mg of plant tissue following a modified CTAB protocol [22]. Lysis was performed by homogenizing liquid nitrogen-ground plant tissues with CTAB buffer containing polyvinylpyrrolidone (PVP) and β-mercaptoethanol. After a 30-minute incubation at 65°C with constant shaking, chloroform extraction was performed. RNA was precipitated overnight with lithium chloride at 4°C, pelleted by centrifugation, washed with 70% ethanol,

air-dried, and dissolved in nuclease-free water. RNA integrity was assessed on 1.5% agarose gels, and concentrations were measured using a NanoDrop One spectrophotometer (Thermo Fisher Scientific).

All reverse transcription reactions were performed in 15 μL volumes containing oligo(dT) and random hexamer primers, dNTPs, and RevertAid Reverse Transcriptase (Thermo Fisher Scientific). Pathogen-specific primers for PVY, PLRV, PVM, and PSTVd detection were used for PCR (Table 3).

Table 3

Specific primers for pathogen detection

Primers for Detection	Forward Primer Sequence (5'→3')	Reverse Primer Sequence (5'→3')	Source
PVM (202 bp)	CGTACAACAGGCCGTCCAT	CGCGGATCCAGGAATACGG	[22]
PLRV (249 bp)	GCCGCTCAAGAAGACTGGAG	GGGGGTCCAACCTATAACGGAT	[22]
PVY (535 bp)	GCATCCAGTCAAACCGGAAC	GCATACGCGCTCTAACCCAC	[22]
PSTVd (123 bp)	ACCCTTCCTTTCTTCTGGGTG	GAAAAGGCGGTTCTCGGGAG	[23]

Virus and Viroid Inoculation

Potato plants were inoculated with the following pathogens: Potato leafroll virus (PLRV), Potato virus Y (PVY), Potato virus M (PVM), and Potato spindle tuber viroid (PSTVd). Infections were carried out both individually and in combination, including six different combinations (PVY + PVM, PVM + PLRV, PVY + PVM + PSTVd, PLRV+PVY, PSTVd+PVY, PLRV+PVY+PSTVd), to clearly determine any synergistic or antagonistic effects.

For mechanical inoculation, infected tissue (0.01 g) was homogenized in 5 mL of 0.06 M phosphate buffer (pH 7.0). To facilitate pathogen entry, an abrasive material was applied, and the leaves were lightly rubbed before applying the homogenate. Plants were grown under a 16-hour light/8-hour dark photoperiod at 18–25°C. Infection symptoms were monitored twice a week for at least three weeks post-inoculation [23].

CRISPR/Cas Constructs

Bioinformatic tools were used to identify conserved genomic regions of the viruses and viroids suitable for targeting by the guide RNA (gRNA) sequences. Furthermore, the first RNA sequences developed to induce RNA interference were sense and antisense. The sequences were synthesized and cloned into pBluescript II KS(+) intermediate vectors driven by particular promoters (AtU6 for gRNA and CaMV 35S for sense and antisense sequences). Cas13d self-fused to GFP subcloned into the binary vector pCambia2300.

Restriction enzymes XmaI and XbaI were used to digest the intermediate constructs to facilitate ligation into binary vectors. The recombinant constructs were initially transformed into E. coli DH5α for plasmid amplification and later electroporated into Agrobacterium tumefaciens EHA105 using Gene Pulser Xcell (Bio-Rad) (Table 4).

Table 4

Constructs used for agroinfiltration of infected plants

Variant number	Genetic constructs for agroinfiltration carrying guide RNA, sense, and antisense sequences	Viruses individually and in combinations for plant inoculation
1	PVY_T1_T2	PVY
2	PVY_S_AS	
3	PLRV_T1_T2	PLRV
4	PLRV_S_AS	
5	PVM_T1_T2	PVM
6	PVM_S_AS	
7	PSTVd _T1_T2	PSTVd
8	PVM_T1_T2 +PLRV_T1_T2	PVM + PLRV
9	PLRV_S_AS + PVM _S_AS	
10	PLRV_T1_T2 + PVY_T1_T2	PLRV + PVY
11	PLRV_S_AS+ PVY_S_AS	
12	PVY_T1_T2 +PVM_T1_T2	PVY+ PVM
13	PVY_S_AS +PVM_ S_AS	
14	PSTVd_T1_T2 + PVY_T1_T2	PSTVd + PVY
15	PVM_T1_T2 +PVY_T1_T2+PSTVd_T1_T2	PVM + PVY + PSTVd
16	PLRV_T1_T2 + PVY_T1_T2+PSTVd _T1_T2	PLRV + PVY + PSTVd

Agroinfiltration

Agrobacterium suspensions were made in infiltration buffer (10 mM MgCl2, 10 mM MES, 200 μM acetosyringone), and the OD600 was diluted to 0.5. Agroinfiltration was performed by injecting the suspensions into the intercellular spaces of leaves using a needleless syringe. Young plants with 5–6 leaves were selected, and four opposite-positioned leaves were infiltrated per plant. Constructs targeting individual pathogens and combinations were tested. Plants were grown under the same conditions as inoculated plants, and leaf samples were collected 3–5 days post-infiltration.

Protein Expression Analysis

Fluorescence microscopy and Western blotting confirmed the expression of Cas13d-GFP fusion proteins. Fluorescence imaging was performed using an EVOS™ M5000 Imaging System with corresponding filters and objectives (10x, 20x, 40x). The proteins were extracted with standard lysis buffer, separated using 7.5% SDS-PAGE gels, and transferred to PVDF membranes by semi-dry blotting. Primary monoclonal anti-GFP antibodies (Santa Cruz Biotechnology) and secondary goat anti-mouse antibodies were used to detect GFP-tagged proteins. Blots were developed with NBT/BCIP substrates.

qPCR Analysis

The expression levels of gRNA, sense, and antisense RNA, and viral RNA loads were quantified using qPCR. Reactions were performed in 20 µL volumes with Luna Universal qPCR Master Mix (NEB) and primers specific to each target (Tables 5 and 6). Thermal cycling was conducted on a QuantStudio 5 system with an initial denaturation step (95°C, 3 min), followed by 40 cycles of denaturation (95°C, 15 s), annealing (60°C, 20 s), and extension (72°C, 40 s). Three biological replicates with technical duplicates were analyzed for each construct and pathogen combination. Relative expression levels and viral loads were calculated using the ΔΔCt method, normalized to GAPDH as an internal control.

Statistical Analysis

Data were analyzed using the R statistical programming environment. Paired t-tests were employed to compare viral loads and construct expression levels between treated and control plants. Significance was set at $p < 0.05$. Statistical summaries were generated using the ggplot2 and dplyr packages in R.

Table 5

Sequences of specific primers for confirming gRNA expression, sense, and antisense sequences

Primer Name	Primer Sequence (5'→3')
PVM-gRNA-1-f	AACCCCTACCAACTGGTC
PVM-gRNA-probe	TTGAAACACCAAATACACATACCATCCA
PVM-gRNA-1-r	TGGAGTGATTGATAGATTGTTG
PLRV-gRNA-1-f	AACCCCTACCAACTGGTC
PLRV-gRNA-probe	AACTATTCCATCCTTGAATGCCGGA
PLRV-gRNA-1-r	GTCCGGCATTCAAGGATG
PVY-gRNA-1-f	AACCCCTACCAACTGGTC
PVY-gRNA-probe	AACTATTCCATCCTTGAATGCCGGA
PVY-gRNA-1-r	GTCCGGCATTCAAGGATG
PVY-gRNA-1-f	AACCCCTACCAACTGGTC
PVY-gRNA-probe	GGGTTTGAAACGTGAAACCCCTGTTTC
PVY-gRNA-1-r	TCCCGCGGAAACAGGG
PVM_as_f	GTAGCTTAATTCGCAGATTG
PVM_as_probe	GACAGTGCGTCTCTGGCATA
PVM_as_r	CTTGATCCCAATTCCCACTCA
PVY_as_f	ATTTGCTTGAGTATGTCCA
PVY_as_probe	CGGATGGCAATCGACATAGG
PVY_as_r	ATTCATCACAGTTGGCATCT
PLRV_as_f	CCAGAGGAAACAAAGTGATCA
PLRV_as_probe	TGCAAGGCTCCATAGAGACA
PLRV_as_r	GGAGGCATTGTCAGAAATG

Results Validation

Construct efficacy was assessed by analyzing viral RNA suppression, symptom alleviation, and construct expression levels. Constructs with sense and antisense sequences exhibited superior RNA interference activation, while gRNA-Cas13 combinations effectively targeted viral RNA, demonstrating the potential of this dual strategy for pathogen control.

Table 6

Specific primer sequences for viral pathogens and viroid

Pathogen Name	Detection Primers	Primer Sequence (5'→3')	Source
PLRV	PLRV-F PLRV-R PLRV probe	5'AAAGCCGAAAGGTGATTAGGC3' 5'CCTGGCTACACAGTCGCGT3' 5'/Cy5/CTCAACGCCTGCTAGAGACCGTCGAAA/BHQ-2/3'	[23]
PVY	PVY – F PVY – R PVY probe	5'GGGTTTAGCGCGTTATGCC3' 5'TCTTGTGTACTGATGCCACCG3' 5'/HEX/ CAGTGAGGGCTAGGGAAGCGCACA/BHQ-1/3'	
PVM	PVM – F PVM – R PVM probe	5'CCATAGAAGCTCTCAGCCGG3' 5' TTCATGCCACCAGTGACCTC3' 5'/6-FAM/ ATACTGCTGCAGTCCAACCC/BHQ-1/3'	
PSTVd	PSTVd – F PSTVd[26] – R PSTVd probe	5'GCCGAAACAGGGTTTTCACC3' 5'GTTTCCACCGGGTAGTAGCC3' 5'/6-FAM/ TTCTTCGGGTGTCCTTCCTC/BHQ-1/3'	
GAPDH (internal control)	GAPDH – F GAPDH – R GAPDH probe	5'TAGCTGCACCACTAACTGCC3' 5'TGCCTTCGGATTCCTCCCTA3' 5'/6-FAM/ TTCCGTGTCCCAACCGTTGA/BHQ-1/3'	

Results

Suppression of viral load using RNAi and CRISPR/Cas13 constructs

Significantly lower levels of viral RNA were detected in potato plants treated with RNAi and CRISPR/Cas13-based constructs (Table 7). Constructs targeting PVY (PVY_T1_T2) achieved an 85% reduction in viral RNA as measured by qPCR (ΔCt = -3.2). Similarly, PLRV_T1_T2 and PVM_T1_T2 constructs resulted in 78% and 82% reductions in viral RNA levels, respectively. Dual-target constructs such as PLRV_S_AS + PVY_S_AS exhibited synergistic effects, with viral load reductions exceeding 90% ($p < 0.001$). Combining RNAi and CRISPR/Cas13 increases efficiency of viral suppression (4), according to data (Figure 1).

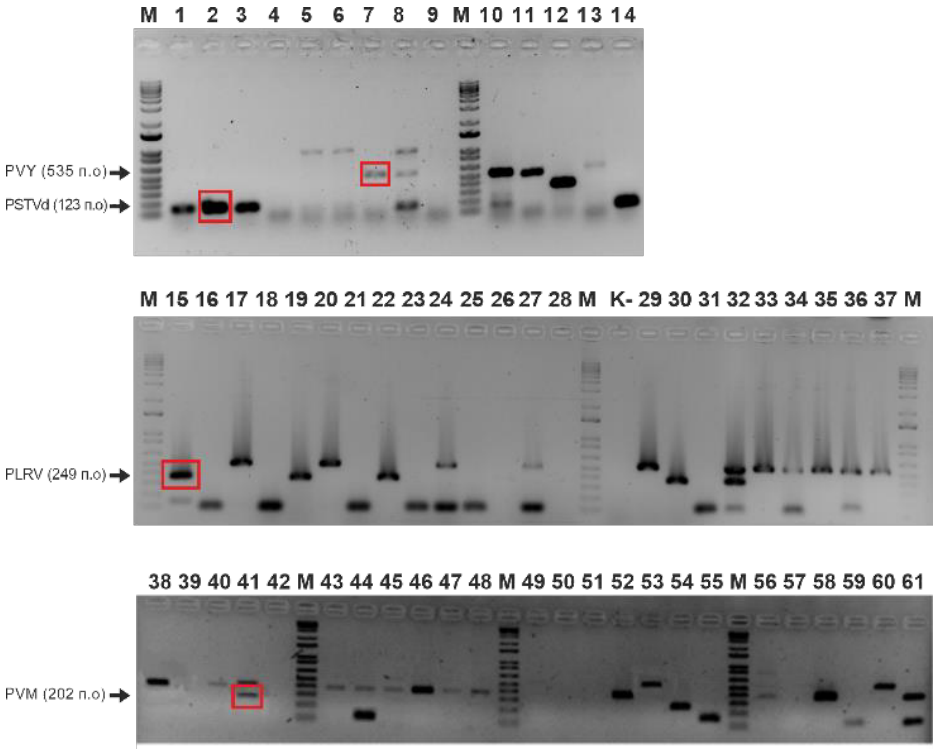
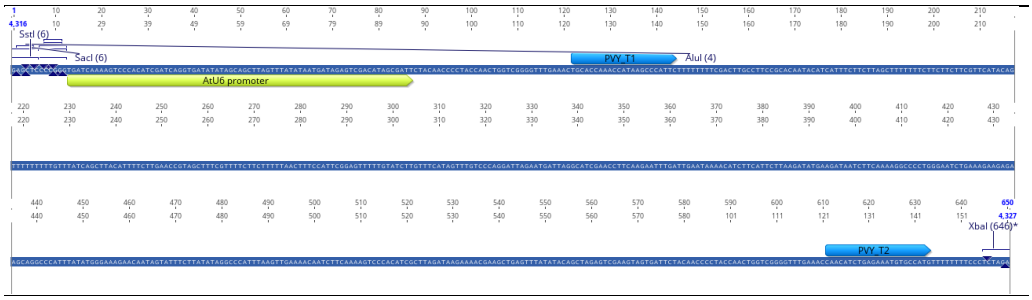
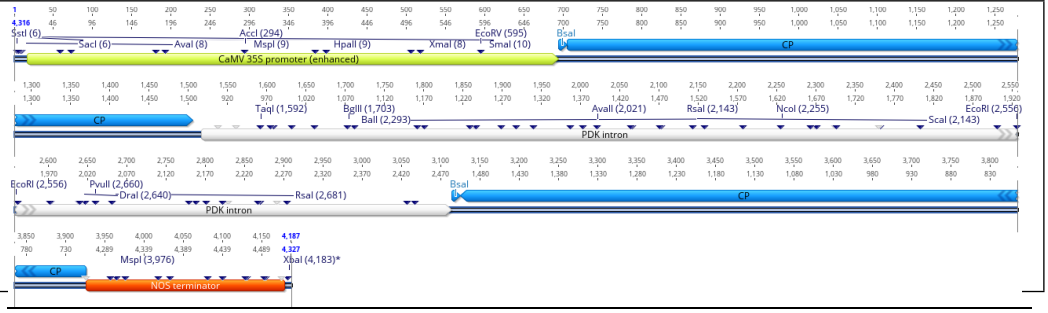
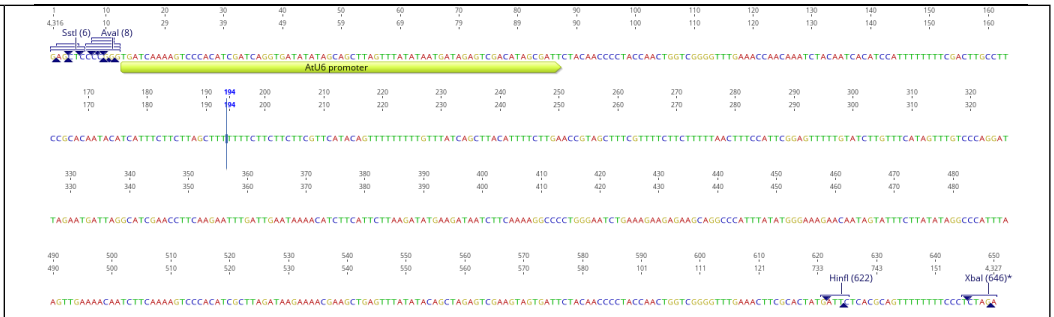
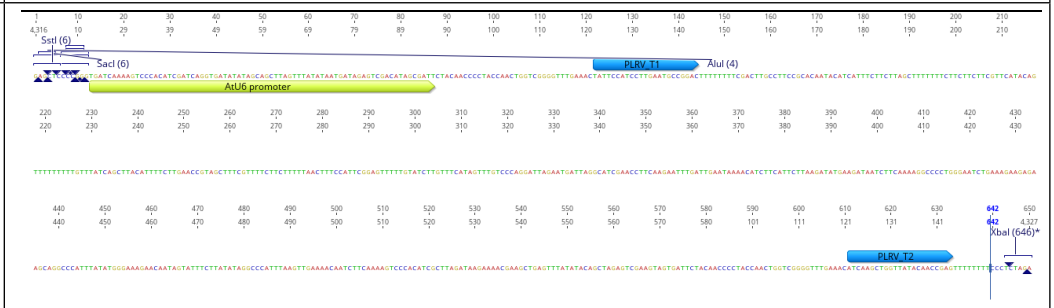
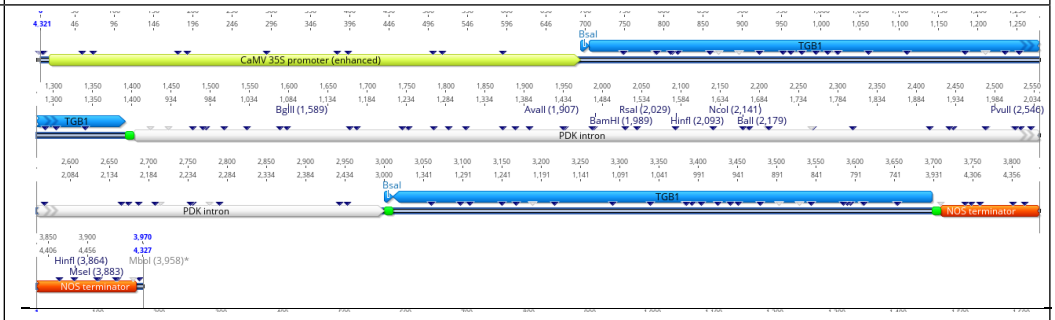
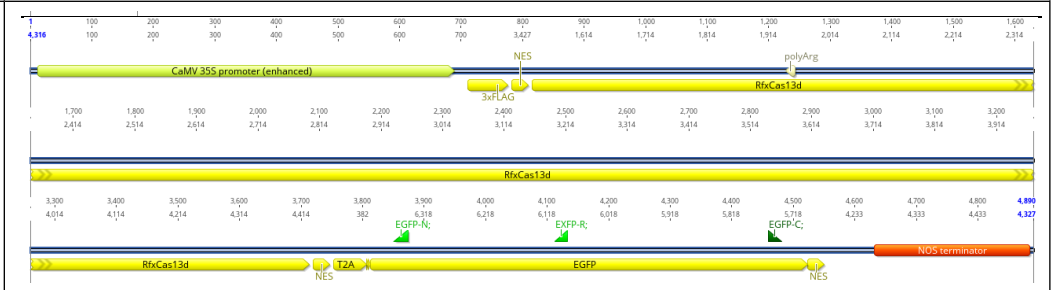


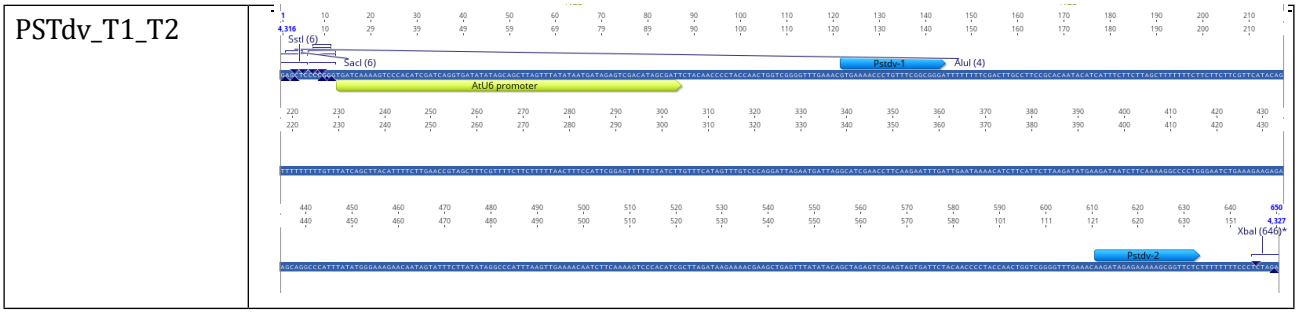
Figure 1. Detection of PVY, PLRV, PVM viruses, and PSTVd viroid in potato samples after inoculation

Table 7

Constructs Carrying Guide RNA, Sense and Antisense Sequences, and the Cas13 Gene

Name of Genetic Constructs	Schematic Representation
PVY_T1_T2	
PVY_S_AS	

PVM_T1_T2	
PLRV_T1_T2	
PLRV_S_AS	
PVM_ S_AS	
Cas13d_GFP	



Validation of Cas13d-GFP Expression and Localization

The expression and functionality of the Cas13d-GFP fusion protein were validated through fluorescence microscopy and Western blotting (Figures 2, 3). Fluorescence microscopy confirmed the proper cytoplasmic localization of the fusion protein, while Western blot analysis showed clear protein bands corresponding to Cas13d-GFP at the expected molecular weight. These results confirm efficient expression and retention of functionality in treated potato plants.

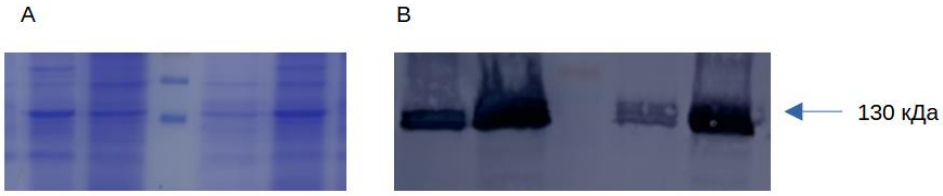


Figure 2. Analysis of Cas13d Protein Expression: (A) Results of Cas13d Protein Electrophoresis in Polyacrylamide Gel; (B) Immunoblotting of Cas13d Protein Using Antibodies to the 3xFLAG Tag

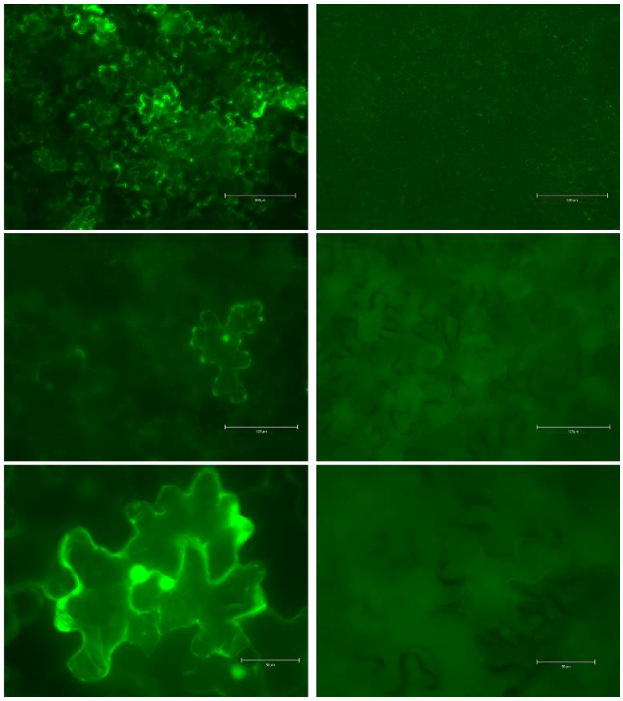


Figure 3. Results of Fluorescence Microscopy: Analysis of eGFP Protein Expression

Alleviation of Disease Symptoms

Plants that received the CRISPR/Cas13 constructs showed a significant reduction of disease symptoms. For instance, PVY-infected plants exhibit less necrosis and leaf deformation, while PLRV-infected plants exhibit minimized leaf curling and chlorosis. Multi-target constructs (e.g., PLRV + PVY + PSTVd) demonstrated the most significant impact, almost fully eliminating symptoms and restoring plant health. These results highlight the therapeutic potential of CRISPR/Cas13 and RNAi technologies for use against viral diseases.

Comparative efficacy of RNAi and CRISPR/Cas13 systems

The study revealed differences in the onset of viral suppression between RNAi and CRISPR/Cas13 constructs. CRISPR/Cas13 constructs as well as RNAi constructs were able to detectably suppress viral RNA, but were faster, with suppression detectable within seven days post-treatment for CRISPR/Cas13 constructs compared with ten days post-treatment for RNAi constructs (Figure 4). Furthermore, CRISPR/Cas13 exhibited higher specificity in targeting conserved viral regions, reducing the risk of pathogen escape through mutations. These findings position CRISPR/Cas13 as a more precise and efficient tool for pathogen control.

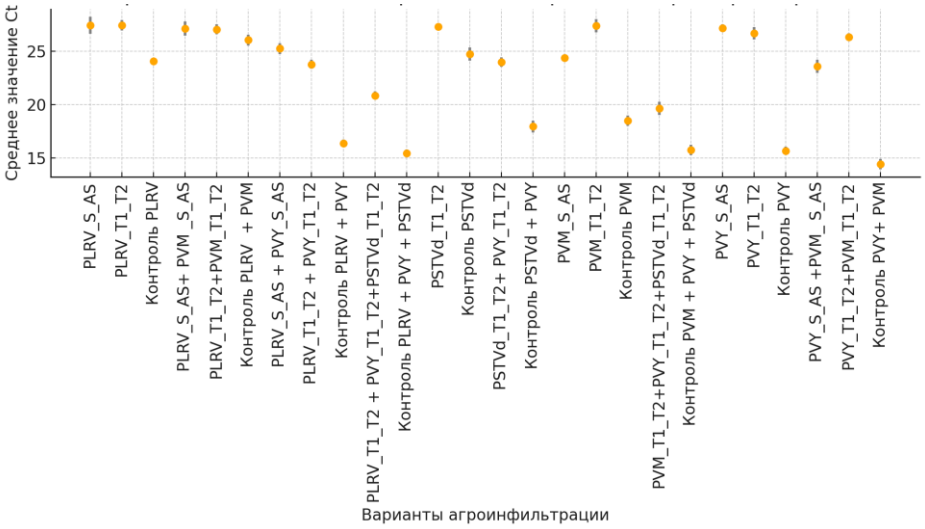


Figure 4. Amplification of Potato Viruses in Plants Expressing gRNA, Sense, and Antisense Sequences

Statistical Analysis and Construct Validation

Statistical analysis in R confirmed that all of the constructs tested led to significantly reduced viral loads as compared to untreated controls ($p < 0.05$). Single pathogen-specific constructs showed, while multi-pathogen constructs provided comprehensive suppression with high reproducibility. In addition, paired t-test and other statistical tools were used, so that the experimental results were reliable.

Discussion

The results indicate the potential of CRISPR/Cas13 and RNAi technologies as efficient tools for controlling viral infection in potatoes. The rapid action, specificity, and scalability of CRISPR/Cas13 systems make them a promising alternative to traditional approaches. The combinations of RNAi and CRISPR/Cas13 strategies pave the way for the generation of transgene-free and sustainable solutions for potato crop protection.

Further research should focus on optimizing guide RNA design, improving the durability of these systems under field conditions, and scaling up for broader agricultural applications. Such progress may also help secure global food supplies by preventing losses from viral pathogens in potatoes and other staple crops [24-30].

Conclusion

RNAi and CRISPR/Cas13 systems are effective for the control of important potato viruses. The constructs greatly reduced viral RNA levels, improved disease symptoms, and took the first steps toward effective and long-term viral control. CRISPR/Cas13 exhibited faster action and greater specificity compared to RNAi, making it a promising tool for the development of transgene-free crop protection strategies. Future research should focus on optimizing these technologies for field applications, paving the way for enhanced resilience in potato production and improved global food security.

Author Contributions

D.G – concept and supervision of the work; **K.A., A.K. –** conducting the experiments; **K.A., R.K. –** discussion of the research results; **K.A., R.K. –** writing the text; **D.G. –** editing the text of the article.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

Compliance with ethical standards

This article does not contain a description of studies performed by the authors involving people or using animals as objects.

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Индукцияланған РНҚ интерференциясы және оның өсімдіктердегі картоп вирусының амплификациясына әсері

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Аңдатпа. Картоп дақылдарындағы вирустық аурулар, әсіресе картоптың Y вирусы (PVY), картоп жапырағының шиыршық вирусы (PLRV), картоптың M вирусы (PVM) және картоп шпинделінің түйнегі вироиды (PSTVd), бақылауға ерекше қиындық туғызады және әлемдік ауыл шаруашылығы

үшін маңызды мәселе болып табылады. Осы зерттеуде біз РНҚ-интерференциясы (RNAi) және CRISPR/Cas13 технологияларының вирустық титрлерді төмендету және патогенездің алдын алу қабілетін бағалаймыз. Картоп өсімдіктері жеке және біріктірілген вирустық қоздырғыштармен жұқтырылды, содан кейін вирустық РНҚ-ның RNAi және Cas13 арқылы деградациясы үшін gРНҚ, сенсорлық және антисенс тізбектерін қамтитын конструкциялармен өңделді. Вирустық РНҚ деңгейін өлшеу үшін сандық ПТР (qPCR) қолданылды, ал аурудың дамуы үш апта бойы бақыланды. Cas13 экспрессиясы флуоресцентті микроскопия және Western blot әдістерімен расталды. RNAi конструкцияларының нәтижелері PVY үшін вирустық РНҚ деңгейін 85%-ға, ал PLRV үшін 78%-ға дейін төмендетті. CRISPR/Cas13 негізіндегі конструкциялар одан да жоғары басу деңгейін көрсетті (кейбір емдеу нұсқаларында ≥90%) және симптомдардың айтарлықтай жеңілдеуімен ерекшеленді. Бірнеше мақсатты бағыттаған конструкциялардың синергиялық әсерлері вирустық жүктеменің ең үлкен төмендеуімен және ауру ауырлығының айтарлықтай азаюымен байқалды. Өңделген және бақылау топтарындағы өсімдіктер арасындағы айырмашылықтар статистикалық тұрғыдан маңызды болды ($p < 0,05$). Бұл зерттеу RNAi және CRISPR/Cas13 технологияларының картоптың вирустық инфекцияларға төзімділігін арттыра алатынын көрсетеді. Бұл әдістер ауруларды бақылауға арналған кеңінен қолдануға болатын, трансгенсіз тәсілді қамтамасыз етіп, тұрақты ауыл шаруашылығына және жаһандық азық-түлік қауіпсіздігіне үлес қосады.

Түйін сөздер: картоп вирусы, РНҚ-интерференциясы, CRISPR/Cas13, гендерді редакциялау, вирус басу, молекулалық құралдар, тұрақты ауыл шаруашылығы, трансгенсіз технология

Индуцированная РНК-интерференция и её влияние на амплификацию вируса картофеля в растениях

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Аннотация. Вирусные заболевания картофеля, особенно вирус Y картофеля (PVY), вирус скручивания листьев картофеля (PLRV), вирус M картофеля (PVM) и вироид веретеновидности клубней картофеля (PSTVd), крайне трудно контролировать, и они представляют собой серьезную проблему для мирового сельского хозяйства. В этом исследовании мы оцениваем противовирусный потенциал технологий РНК-интерференции (RNAi) и CRISPR/Cas13 в снижении вирусных титров и предотвращении патогенеза. Растения картофеля были инфицированы как отдельными, так и комбинированными вирусными патогенами, а затем обработаны конструкциями, содержащими гайдовую РНК (гРНК), сенсорные и антисмысловые последовательности, используемые как для RNAi, так и для деградации вирусной РНК, опосредованной Cas13. Количественная ПЦР (qPCR) использовалась для измерения уровней вирусной РНК, а прогрессирование заболевания наблюдалось в течение трех недель. Экспрессия Cas13 была подтверждена с помощью флуоресцентной микроскопии и вестерн-блоттинга. Результаты экспериментов с конструкциями RNAi показали снижение уровня вирусной РНК

до 85% для PVY и 78% для PLRV. Конструкции CRISPR/Cas13 продемонстрировали еще более высокие показатели подавления вирусов (≥90% в некоторых вариантах обработки) с заметным облегчением симптомов. Синергетический эффект конструкций, направленных на несколько мишеней, привел к максимальному снижению вирусной нагрузки и тяжести заболевания. Различия между обработанными и контрольными растениями были статистически значимыми ($p < 0,05$). Это исследование демонстрирует, что RNAi и CRISPR/Cas13 могут повысить устойчивость картофеля к вирусным инфекциям. Обеспечивая масштабируемый, трансгенез-необусловленный подход к контролю заболеваний, эти методы способствуют развитию устойчивого сельского хозяйства и обеспечению глобальной продовольственной безопасности.
Ключевые слова: вирус картофеля, РНК-интерференция, CRISPR/Cas13, редактирование генов, подавление вирусов, молекулярные инструменты, устойчивое сельское хозяйство, трансгеннезависимая технология

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Розанная листовертка *Archips rosana* L. как вредитель яблони Сиверса в Илейском Алатау

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Аннотация. Данная статья содержит новые сведения по фенологии вида *Archips rosana* L. Целью исследования является определение фенологических особенностей и выявление уязвимых стадий развития розанной листовертки. Практическая значимость заключается в том, что данные исследования позволят более подробно выяснить пути экологической адаптации этого вида к местным условиям обитания, которая в последующем способствует управлению популяциями данного насекомого-вредителя. Исследования были проведены на территории Иле-Алатауского государственного национального природного парка. Материал был получен авторами в течение двух полевых сезонов 2018-2019 гг. Методами исследования являются классические энтомологические методы, такие, как отлов самцов и самок с помощью энтомологического сачка, полустационарный учет на мониторинговых площадках, также выращивания листоверток методом культивирования личинок в лаборатории энтомологии Института зоологии. В результате исследований были уточнены фенологические особенности розанной листовёртки в Илейском Алатау. Исследовано влияние температуры и относительной влажности воздуха (%) на развитие розанной листовертки *Archips rosana* L. в 2018 и 2019 году. В ходе исследования были выявлены уязвимые стадии развития – стадия гусениц 1-2 возраста, которые появляются на исследуемых территориях со второй декады апреля до первой декады мая.

Ключевые слова: яблоня Сиверса, розанная листовертка, *Archips rosana* L., насекомые-вредители, Илейский Алатау

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Введение

Цель исследования – определение фенологических особенностей и выявление особо опасных периодов развития розанной листовертки. Актуальность исследования заключается в необходимости защиты яблони Сиверса от насекомого-вредителя розанной листовертки *Archips rosana* L.

Яблоня Сиверса *(Malus sieversii)* является одним из основных лесообразующих дикорастущих видов в горных экосистемах юга и юго-востока Казахстана [1-4]. Современные генетические исследования (примерно 2500 современных сортов яблонь) показали, что яблоня Сиверса является прародительницей практически всех сортов домашней яблони [5-6]. В настоящее время насекомые-вредители стали одной из основных угроз для диких популяций яблони Сиверса, которые наносят огромный урон дикорастущим плодовым лесам в Казахстане [7-9].

Стоит отметить, что среди насекомых-вредителей семейство листовертоk *(Tortricidae)* из отряда чешуекрылых *(Lepidoptera)* занимает особое место [10]. Листовертки получили своё название из-за того, что гусеницы большинства видов живут в свёрнутых листьях, повреждают и часто скелетируют их [11-12]. Они представляют серьёзную опасность в энтомоценозах не только культурных садов и природных популяций плодовых лесов, но и в плодово-ягодных питомниках. Особую опасность для яблони представляют гусеницы розанной листовертки, которые в течение вегетационного периода могут нанести плодовым деревьям непоправимый ущерб, повреждая цветочные почки (бутоны), сочные листья и плоды [13-15]. Ощутимый урон личинки этой листовертки причиняют молодым плодовым деревьям, поскольку обгладывают верхушки зеленых побегов в период формирования кроны [18].

При массовой вспышке численности гусеницы могут погубить основную часть урожая, потому что при появлении первых цветочных завязей гусеницы почти полностью выедают соцветия и цветоножки. После того, как плодовые деревья отцветут, гусеницы переключаются на листья, нарушая, таким образом, нормальный процесс фотосинтеза, а с появлением плодов поселяются внутри них, делая их непригодными для употребления [19]. Этот вид насекомого-вредителя при массовой вспышке численности может поражать от 30 до более 50 процентов листьев на отдельных деревьях, что является опасным порогом вредоносности, поскольку существенно снижает качество и количество урожая.

Морфологические и биологические особенности: передние крылья имаго розанной листовёртки имеют разнообразную окраску от охряно-жёлтого до серо- и тёмно-коричневого цвета; задние крылья коричнево-серые, у самок с ярким оранжево-жёлтым опылением у вершины крыла. Размах крыльев обычно 15-22 мм. Размер гусениц 18-20 мм, от светло-зеленой до тёмно-оливково-зелёной окраски, полупрозрачные. Яйца удлиненно-овальные, серо-зеленые. Куколка 10-12 мм, веретеновидная, желтовато-коричневая [20]. Зимуют яйца под корой или на гладкой поверхности коры ветвей, иногда гусеницы зимуют под опавшими листьями. Гусеницы 1-2 возрастов выгрызают почки и бутоны, затем, переходя на цветки, уничтожают тычинки, пестики и лепестки, а также скелетируют молодые листья и стягивают их тонкой паутинкой в комок. Гусеницы 3-5

возрастов повреждают завязи и плоды, выгрызая в мякоти ямки неправильной формы, достигающие иногда семенной камеры или косточки [21]. Лёт бабочек происходит в сумерки. В Илейском Алатау розанная листовёртка развивается в одном поколении.

Розанная листовёртка – широкий полифаг и отмечен на 130 видах кормовых растений из 32 семейств. Распространение очень широкое, вид известен из Европы, Средиземноморья, Ирана, Ирака, Закавказья, Центральной Азии и Южной Сибири, завезён на Дальний Восток и в Северную Америку. Особенно существенный вред розанная листовёртка наносит плодовым и ягодным культурам [22].

Непременным условием успешной защиты от розанной листовертки является установление времени появления уязвимых стадий развития, во время которых следует проводить обработку деревьев от этого вредителя для достижения наибольшего эффекта. В связи с этим для определения сроков таких обработок очень важно установить сроки развития различных стадий этого вредителя, особенно: а) начало массового лета бабочек; б) начало массовой откладки яиц и в) начало массового отрождения гусениц [23].

Материал и методы исследования

Исследования были проведены на территории Иле-Алатауского государственного национального природного парка. Объект исследования – розанная листовертка *Archips rosana* L. как один из доминантных видов насекомых-вредителей яблони Сиверса. Материал для исследований был получен авторами в течение двух полевых сезонов 2018-2019 гг. Методами исследования являются классические энтомологические методы, такие, как отлов самцов и самок с помощью энтомологического сачка, полустационарный учет на мониторинговых площадках, также выращивание листоверток методом культивирования личинок в лаборатории энтомологии Института зоологии. Полустационарный учет проводился на 5 мониторинговых площадках Иле-Алатауского государственного национального природного парка, координаты мониторинговых площадок отражены в таблице 1.

Таблица 1

Географические координаты мониторинговых площадок

№	Расположение	Долгота	Широта	Высота (м)
1	Аксайский филиал, Аксайское лесничество	E - 76º47'58"	N - 43º7'23"	H-1345м
2	Талгарский филиал, Талгарское лесничество	E - 77º21'16"	N - 43º 16´5"	H-1538м
3	Тургенский филиал, Иссыкское лесничество	E - 77º29'05"	N - 43º15'11"	H-1714м
4	Тургенский филиал, генетический резерват «Кузнецово ущелье»	E - 77º40'21"	N - 43º22'05"	H-1595м
5	Талгарский филиал, Котырбулакское лесничество	E - 77º06'57"	N - 43º16'39"	H-1025м

Во время полевых наблюдений и сборов собранные гусеницы помещали в стеклянные пробирки вместе с листьями яблони Сиверса. Эти искусственные садки отмечались порядковыми номерами, под соответствующем номером в журнал заносили сведения о месте и времени сбора, сведения о растении, характере его повреждений и другую информацию. В дальнейшем для линьки и содержания гусеницы пересаживались в отдельные пробирки.

Исследования были проведены согласно общепринятым энтомологическим методикам [16-17]. Температура измерялась полевым термометром. Для определения фенологических фаз личинок всех пяти возрастов были использованы методики измерения головной капсулы и наблюдения как в природе, так и в стационарных садках. Все энтомологические объекты фотографировались и были исследованы под бинокулярным микроскопом KLM-105.

Результаты и обсуждение

В результате исследования выяснилось, что розанная листовертка в условиях Илейского Алатау зимует в фазе яиц, уложенных в один слой группами по примерно 60 (40-100) штук в кладке на гладкой коре в нижней части ствола яблони и в развилках крупных веток. Развитие отложенных в прошлом году яиц происходит в горных условиях на высоте 1200-1600 м над уровнем моря примерно в течение двух недель ранней весной в начале апреля, после чего из них вылупляются личинки первого возраста. В это время (первая декада апреля) при средней суточной температуре воздуха +7+14°С, когда почки яблони появляются, гусеницы первого возраста живут сначала вместе, но затем расползаются на верхушки побегов. Листовые сверточки являются явным признаком наличия вредителя. Если к гусенице дотронуться, она не упадет на землю, а повиснет в воздухе на паутинке.

В таблице 2 и рисунке 1 приводятся сведения по фенологическим особенностям розанной листовертки. Первое появление гусениц этого вида отмечено в начале второй декады апреля при среднесуточной температуре воздуха +11°С и относительной влажности воздуха 56 %. Развитие гусениц розанной листовертки происходит здесь в течение примерно 30–40 дней. В течение своего развития гусеницы линяют четыре раза и проходят пять возрастов.

Гусеницы продолжают питаться на яблоне до конца мая. По нашим наблюдениям, окукливание гусениц начинается в начале третьей декады мая (в 2019 г. первое окукливание произошло 23 мая при среднесуточной температуре +21°С). В конце мая - начале июня окукливание гусениц принимает массовый характер, процесс развития куколки длится около двух недель при среднесуточной температуре +15 + 25°С, после чего, начиная со второй декады июня, из куколок отрождаются имаго, которые после вылета сразу приступают к спариванию и дальнейшей яйцекладке.

Таблица 2

Ширина головных капсул в зависимости от возраста и продолжительность стадий развития гусениц

Возраст	I	II	III	IV	V
Продолжительность стадий развития, суток	9–12	5–6	5–6	8–10	5–7
Ширина головной капсулы, мм	0,175–0,300	0,325–0,450	0,550–0,775	0,850–1,275	1,325–1,925

Первое появление бабочек отмечено в середине июня при среднесуточной температуре +25+30°С, массовый вылет с третьей декады июня при среднесуточной температуре +28°С. Самки выходят с некоторым количеством созревших яиц. Откладка яиц начинается через 3–5 суток после вылета бабочек с конца июня при среднесуточной температуре +30°С. Одна самка за сезон откладывает от четырехсот до восьмисот яиц.

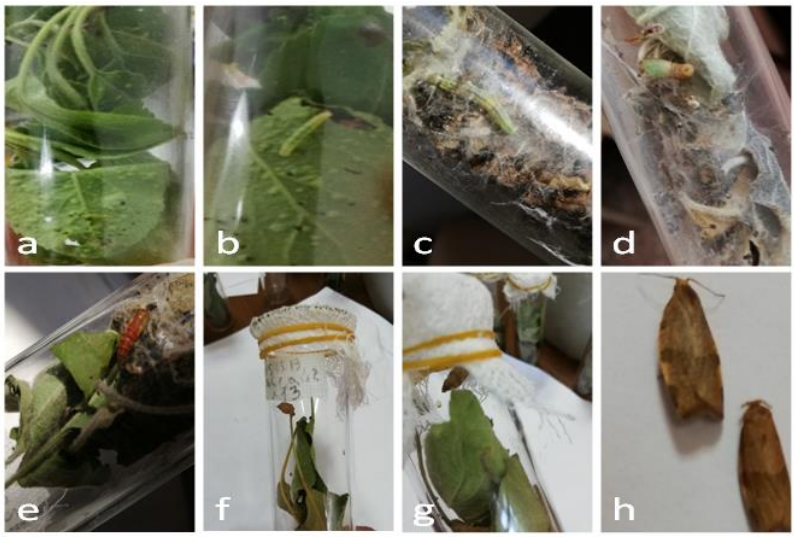

Рисунок 1. Различные стадии развития розанной листовертки, полученные в лабораторных условиях. **a, b, c –** гусеницы, **d, e** – куколки, **f, g, h** – имаго (Фотографии: Гульжанат Танабекова)

Созревание яиц происходит на протяжении всего периода яйцекладки, но откладываются яйца периодически с промежутками в 2–4 суток. Массовая яйцекладка продолжается 15–20 суток с середины июля. Яйца откладываются на гладкие участки коры. В одной яйцекладке в среднем наблюдалось 55 яиц (от 13 до 146 яиц). К концу жизни имаго откладывание количества нежизнеспособных яиц увеличивается, это в основном наблюдаются в мелких кладках. Яйца в кладке имеют темный цвет и располагаются в области развилок ветвей или в расщелинах и углублениях коры.

В таблице 3 указана наиболее уязвимая стадия для борьбы с этим вредителем – стадия гусениц 1-2 возраста, которые появляются в Илейском Алатау со второй декады

апреля до первой декады мая. По нашим наблюдениям, часто массовый лёт бабочек наблюдается после осадков. Имаго листовертки ведут активный ночной образ жизни, вылетая с наступлением сумерек (при температуре воздуха выше пятнадцати градусов тепла), а в светлое время суток находятся в укрытии (размещаясь на стволах или на боковых ветках под листьями) [24, 25].

Таблица 3

Фенограмма развития розанной листовертки в 2018-2019 гг.

Апрель			Май			Июнь			Июль			Август		
I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
0	Ω	Ω	Ω	Ω	Ω □	□	+	+0	+0	+0	+0	+0	0	0
	▲	▲	▲	▲										

Примечание: □ – куколка; + – взрослые вредители; 0 – яйцекладка; Ω – выход личинки; ▲ – уязвимый период.

В таблице 4 приводятся данные по фенологическим наблюдениям розанной листовертки в Илейском Алатау в 2018-2019 гг.

Таблица 4

Фенология розанной листовертки *Archips rosana* L. в Илейском Алатау в 2018-2019 гг.

Фенологические фазы развития розанной листовёртки	Дата		Температурные показатели Т°С		Относительная влажность воздуха, %	
	2018	2019	2018	2019	2018	2019
Начало отрождения личинок первого возраста из перезимовавших яиц	17.04.	15.04.	12	14	67	71
Массовое отрождение личинок 1 возраста и начало активного питания	22.04.	20.04.	13	16	41	68
Гусеницы 2 возраста	03.05.	01.05.	18	20	39	54
Гусеницы 3 возраста	08.05.	06.05.	16	18	29	37
Гусеницы 4 возраста	12.05.	10.05.	23	25	52	45
Гусеницы 5 возраста	20.05.	18.05.	18	20	30	42
Начало окукливания	25.05.	23.05.	19	21	38	43
Массовое окукливание	28.05.	27.05.	20	24	36	30
Начало лёта имаго	03.06.	01.06.	12	27	95	23
Массовый лёт имаго	24.06.	17.06.	25	28	33	28
Начало откладки яиц	30.06.	23.06.	26	29	27	28
Массовая яйцекладка	11.07.	08.07.	28	35	50	30
Начало диапаузы	23.07.	11.07.	30	33	41	39

Длительность различных стадий развития розанной листовертки *Archips rosana* L. в Илейском Алатау по наблюдениям в 2018-2019 гг.: полный жизненный цикл – 1 год (одно поколение), яйцо – 9-10 месяцев, личинки 5 возрастов – 30-40 дней, куколка – 10-14 дней, имаго – более 2 месяцев [26].

Зависимость влияния показателей температуры на фенологию розанной листовертки *Archips rosana* L. в Илейском Алатау в 2018-2019 гг. представлена ниже в виде графика (Рис. 2).

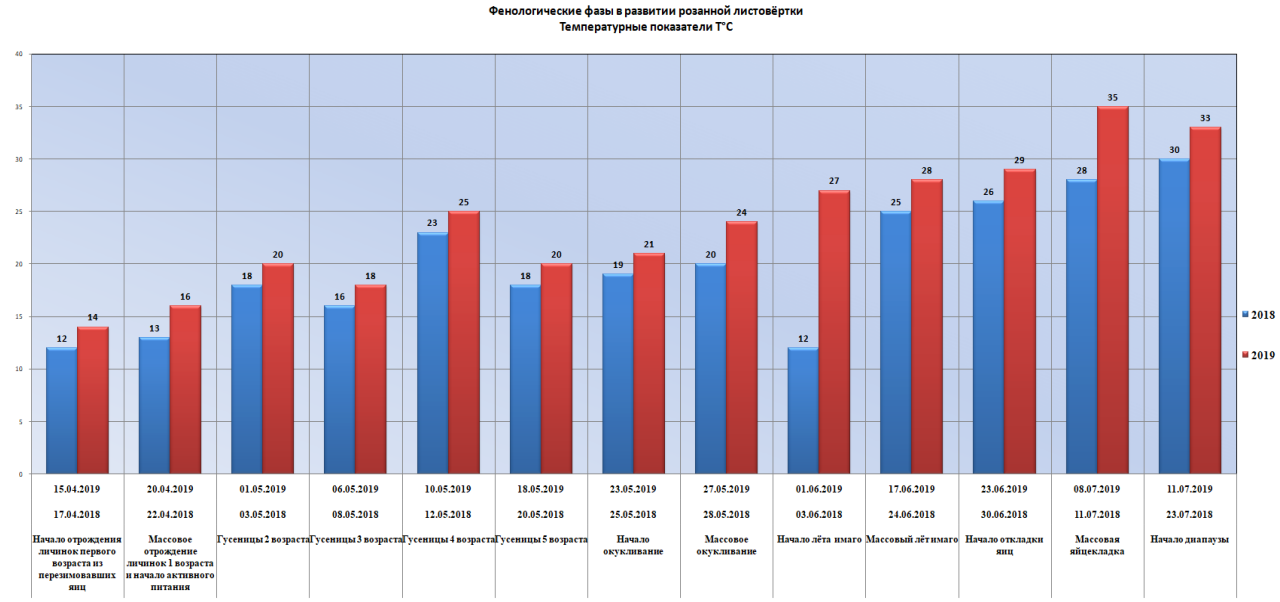

Рисунок 2. Влияние температуры (°C) на фенологию розанной листовертки *Archips rosana* L. в 2018-2019 гг.

В рисунке 2 показано влияние температуры (°C) на фенологию розанной листовертки *Archips rosana* L. в 2018-2019 гг. По результатам двухгодичных исследований были выявлены различия в фенологии розанной листовертки. Начало отрождения личинок первого возраста из перезимовавших яиц в 2018 году было зафиксировано 17 апреля при 12°C, а в 2019 году 15 апреля при 14°C. Массовое отрождение личинок 1 возраста и начало активного питания в 2018 году было зафиксировано 22 апреля при 13°C, а в 2019 году 20 апреля при 16°C. Начало окукливания в 2018 году наблюдалось 25 мая при 19°C, а в 2019 году 23 мая при 21°C. Начало лёта имаго было зафиксировано 3 июня при 12°C, а в 2019 году 1 июня при 27°C. Начало откладки яиц было зафиксировано 30 июня при 26°C, а в 2019 году 23 июня при 29°C.

В рисунке 3, 4 отражено влияние относительной влажности воздуха (%) на развитие розанной листовертки в 2018 и 2019 годах. Начало отрождения личинок первого возраста из перезимовавших яиц в 2018 году было зафиксировано 17 апреля при 67%, а в 2019 году 15 апреля при 71%. Массовое отрождение личинок 1 возраста и начало активного питания в 2018 году было зафиксировано 22 апреля при 41%, а в 2019 году 20 апреля при 68%.

Рисунок 3. Влияние относительной влажности воздуха (%) на развитие розанной листовертки в 2018 году

Рисунок 4. Влияние относительной влажности воздуха (%) на развитие розанной листовертки в 2019 году

Начало окукливание в 2018 году наблюдалось 25 мая при 38%, а в 2019 году 23 мая при 43%. Начало лёта имаго было зафиксировано 3 июня при 95%, а в 2019 году 1 июня при 23%. Начало откладки яиц было отслежено 30 июня при 27%, а в 2019 году 23 июня при 28%.

Выводы

На территории Иле-Алатауского государственного национального природного парка розанная листовертка развивается в одном поколении. Выход гусениц из зимней спячки в условиях среднегорья начинается при средней дневной температуре +17°С. Стадия куколки длится около 10-14 дней при средней температуре +21°С. Массовое окукливание происходит в третьей декаде мая при среднесуточной температуре 20-25°С. Отрождение имаго происходит с конца мая до начала августа с массовым лётом бабочек во 2-й половине июня при среднесуточной температуре +28°С. Продолжительность жизни имаго от 8 до 30 дней [27]. К началу августа происходит постепенный спад имаго. Начало откладки яиц происходит в третьей декаде при среднесуточной температуре +30°С. Эмбриональное развитие яиц включает в себя три периода: развитие до диапаузы (дифференциация и конденсация зародышевой полосы), которое длится несколько суток; облигатная диапауза, которая длится лето, осень и зиму; и окончательное развитие, которое происходит весной при + 8+13°С, нижний порог развития яиц + 8°С. Развитие яиц в природе заканчивается в апреле.

Вклад авторов

Г.Б.Т. и Р.В.Я. – концепция и руководство работой; **Г.Б.Т. и Р.В.Я.** – проведение экспериментов; **Г.Б.Т. и Р.В.Я.** – обсуждение результатов исследования; **Г.Б.Т. и Р.В.Я.** – написание текста; **Г.Б.Т. и Р.В.Я. –** редактирование текста статьи.

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Конфликт интересов

Авторы заявляют об отсутствии конфликта интересов.

Соблюдение этических норм

Все процедуры, выполненные в исследованиях с участием животных, соответствовали этическим стандартам учреждения, в котором проводились исследования, и утвержденным правовым актам РК и международных организаций.

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Раушан жапырақ ширатқыш көбелек *Archips rosana* L. Іле Алатауындағы Сиверс алмасының зиянкесі ретінде

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Аңдатпа. Бұл мақалада *Archips rosana* L. түрінің фенологиясы туралы жаңа мәліметтер қамтылған. Зерттеудің мақсаты – фенологиялық ерекшеліктерін анықтау және раушан жапырақ ширатқыш көбелектің дамуының осал кезеңдерін анықтау. Практикалық маңыздылығы, бұл зерттеулер осы түрдің жергілікті тіршілік ету ортасына экологиялық бейімделу жолдарын егжей-тегжейлі анықтауға мүмкіндік береді, соның негізінде осы зиянкес жәндіктің популяциясын басқаруға ықпал етеді. Зерттеулер Іле-Алатау мемлекеттік ұлттық табиғи саябағының аумағында жүргізілді. Материалды авторлар 2018-2019 жылдардағы екі жылдық далалық маусымда жинады. Зерттеу әдістері – классикалық энтомологиялық әдістер, мысалы, энтомологиялық тордың көмегімен аталықтар мен аналықтарды аулау, бақылау алаңдарында жартылай стационарлық есеп жүргізу, сондай-ақ Зоология институтының энтомология зертханасында дернәсілдерді культивациялау әдісімен өсіру. Зерттеулер нәтижесінде осы аумақтардағы раушан жапырақ ширатқыш көбелектің *Archips rosana* L. фенологиялық ерекшеліктері нақтыланды. 2018 және 2019 жылдары фенологиясына температура мен салыстырмалы ылғалдылықтың (%) әсері зерттелді. Зерттеу барысында дамудың осал кезеңдері анықталды – зерттелетін аумақтарда сәуірдің екінші онкүндігінен мамырдың бірінші онкүндігіне дейін пайда болатын 1-2 жас аралығындағы дернәсілдер кезеңі.

Түйін сөздер: Сиверс алмасы, раушан жапырақ ширатқыш көбелек, Archips rosana L.), жәндік-зиянкестер, Іле Алатауы

Rose leaf roller *Archips rosana* L. as a pest of Sievers apple tree in Iley Alatau

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Abstract. This article contains new information on the phenology of the *Archips rosana* L. The purpose of the study is to determine the phenological features and identify vulnerable stages of rose leaf roller development. The practical significance lies in the fact that these studies will allow us to find out more detailed understanding of the ecological adaptation of this species to local habitat conditions, which subsequently contributes to the management of populations of this insect pest. The research was conducted on the territory of the Ile-Alatau State National Natural Park. The material was obtained by the authors during two field seasons, 2018-2019. The research methods are classical entomological methods, such as trapping males and females using an entomological net, semi-stationary accounting at monitoring sites, and growing leaf rollers by cultivating larvae in the entomology laboratory of the Institute of Zoology. As a result of the research, the phenological features of the rose leaf roller in these territories were clarified. The influence of temperature and relative humidity (%) on the development of the rose leaf roller, *Archips rosana* L., in 2018 and 2019 has been studied. During the study, vulnerable stages of development were identified – the stage of larvae of 1-2 ages, which appear in the studied territories from the second decade of April to the first decade of May.
Keywords: Sievers Apple tree, rose tortrix, *Archips rosana* L., insect pests, the Iley Alatau

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Macrophytes of the Floodplain swamps of the Akmola Region

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Abstract. This paper presents the results of the research on macrophytes of floodplain swamps of the coastal territories of the Aktastinka River and wetlands of Aktasty village, located in Arshalinsky district, Akmola region of the Republic of Kazakhstan. The inventory revealed the floristic composition of the Aktastinka village locality and the coastal territory. Along with species diversity, the main representatives of macrophytes of the bog flora were identified, represented by 8 hydrophytes – *Phragmites australis, Carex rostrata, Typha angustifolia, Ranunculus repens L., Triglochin maritimum L., Stratiotes aloides L., Utricularia vulgaris L., Lemna minor L.*, etc. In the flora of the study area, four types of plant communities were found, with the dominance of representatives of the following families: *Poaceae, Typhaceae, Cyperaceae, Ranunculaceae, Amaryllidaceae, Butomaceae, and Lentibulariaceae* spanning several kilometres. Comparative analysis of macrophyte floras at four different sites illustrated similarity between phytocenoses. The plant flora of the study area includes 105 species belonging to 65 general and 38 families. Dicotyledonous plants are represented by 78 species, monocotyledones plants by 27 species. The ratio of dicotyledons to annuals was 1:2.9. On average, each genus is represented by 2.3 species, species saturation of families is characterised by the average index, and is 9.5.

Keywords: macrophytes, hydrophytes, coastal plants, species diversity, floodplain marshes

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Introduction

The study of the flora of floodplain bogs is currently of great scientific interest. Especially relevant is the study of the species composition of individual, poorly studied areas. One of such areas is the vicinity of Aktasty village. At present, plant communities of river floodplains and floodplain bogs in the Akmola region are practically not studied. Waterlogging is a long-term process occurring under the influence of a number of abiotic, biotic, and anthropogenic factors. Of particular interest were macrophytes of floodplain marshes of the coastal territories of the Aktastinka River and the wetland of Aktasty village, situated in the Arshaly district of Akmola region of the Republic of Kazakhstan. According to the classification of Mirkin B.M., the studied floodplain swamps belong to the type of peaty undeveloped plain floodplains [1-5].

According to the results presented in the studies of a number of authors, soil changes occur in coastal areas during long-term natural processes. The process of waterlogging is affected by both natural and anthropogenic factors. Dynamic observations make it possible to identify the substitution of lowland soils for a waterlogged peat type [6-8].

Macrophytes (higher aquatic and coastal aquatic plants) play a crucial role in aquatic ecosystems in the formation of vegetation cover on the coast of rivers. A comprehensive study of the coastal macrophytes allows us to assess the potential of plants for further use of their indicative properties in biological analyses. The intense adaptive potential of plants allows them to spread over territories with a considerable area, forming coastal populations [9, 10]. The main relationship between aquatic macrophytes and the formation of organic substances, with subsequent formation of bottom sediments, is considered a result of metabolism and reactions at the ecosystem level. The dynamics of the modification of the species composition of macrophytes during aging and waterlogging of the lake is obvious [11-14]. Under the prolonged influence of anthropogenic factors, the cumulative effect of the intake of nutrients in the form of nitrogen and phosphorus exerts pressure on coastal zones, resulting in eutrophication [15-16].

According to the information presented in the studies of various authors, it was found that chemical factors had a greater effect on flooded macrophytes, while the composition of growing macrophytes and macrophytes with floating leaves was best explained by land use factors. The results of this study confirm the use of macrophyte communities as effective indicators of the ecological state of reservoirs [17-19].

For the first time, we carried out integrated studies of floodplain bogs of the coastal territories of the Aktastinka River and wetlands of Aktasty village, which enabled us to expand botanical knowledge, obtain data on taxonomic and cenotic diversity of the vegetation cover of floodplain bogs. A comprehensive study of both coastal and submerged macrophytes allows them to be used as natural bioindicators of the state of reservoirs. This approach is widely used in different countries [20-22]. Our research aimed to study species diversity of the vegetation cover and to identify the composition of the flora of vascular plants of the floodplain bogs of the Aktastinka River and wetlands in the vicinity of Aktasty village.

Materials and research methods

The research objects were macrophyte plants growing in the floodplains of the Aktasinka River, as well as in wetlands in the vicinity of Aktasty village. Location geodata: Kazakhstan, Akmola region, Arshaly district, Aktasty village 50.752216, 72.211137 (Figure 1).

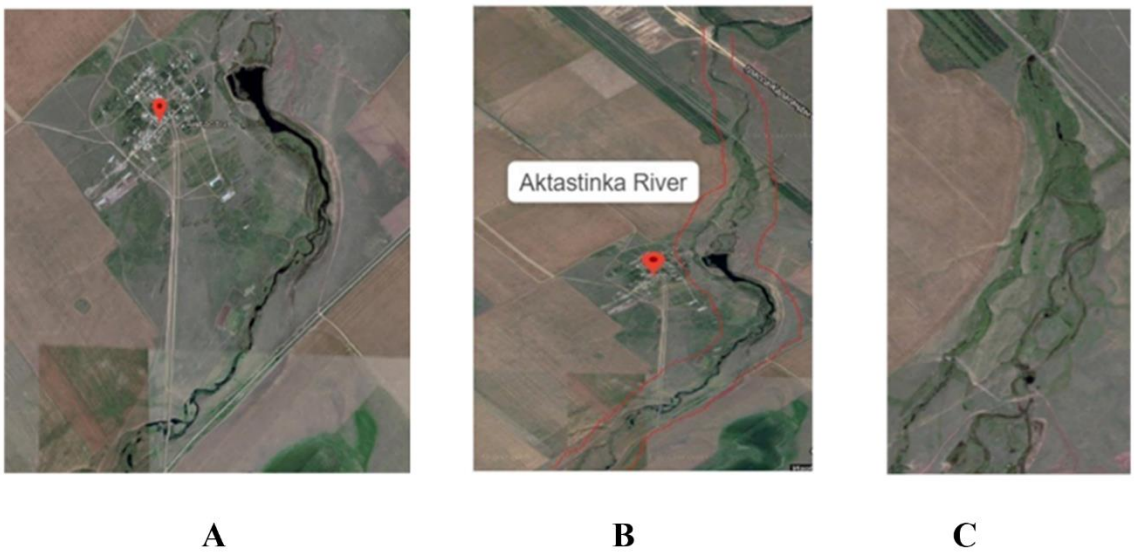

Figure 1. Satellite images: **A –** Aktasty village; **B –** Aktastinka river**; C –** Swampy area №1

Research methods

The flora composition was analyzed during expedition trips in spring and summer during 2023-2024. Field studies were conducted by route-reconnaissance and semi-stationary methods described in the methodological instructions of Darabayeva B.E. et al [23]. During the expeditions, plants were identified, and herbarium specimens were collected to clarify the species affiliation of macrophytes. The study was guided by multi-volume identifiers and well-known summaries [24-25]. The species affiliation of macrophytes of floodplain bogs were determined by using locally assigned identifiers [26-27]. Sample plots were laid in the most homogeneous and typical areas of facies [28]. The description of bog plots was carried out within natural boundaries; the size of the areas was 10 x 10 m. Areas № 1-4 were investigated by laying out profiles, and route diagrams were drawn up. In determining species affiliations, the study was coordinated by well-known identifiers and multi-volume summaries. The area and place of occurrence of each species were clarified based on our field study using satellite images and Google Maps.

The work uses statistical analysis methods to assess the species and taxonomic diversity of macrophytes. Shannon and Simpson indices for biodiversity characteristics, as well as the coefficient of species saturation of genera and families, are calculated. Phytocenoses were compared using cluster analysis, which took into account the degree of their similarity.

Results

Aktasty village is located in the east of Astana city, 60 km away. It belongs to Arshaly district, Akmola region, bordering Ermentau district to the east, Karagandy region to the south, and the city of Astana to the west and north. The village lies within a dry-steppe zone with a continental climate. Winters are cold and prolonged, with an average January temperature of -17°C, while summers are moderately hot, averaging 20°C in July. The average annual precipitation is 300-350 mm. The Aktastinka River flows nearby, discharging into the Ishim River, with small branches, gullies, and ravines along its course. Swampy areas are also present (Figure 2).

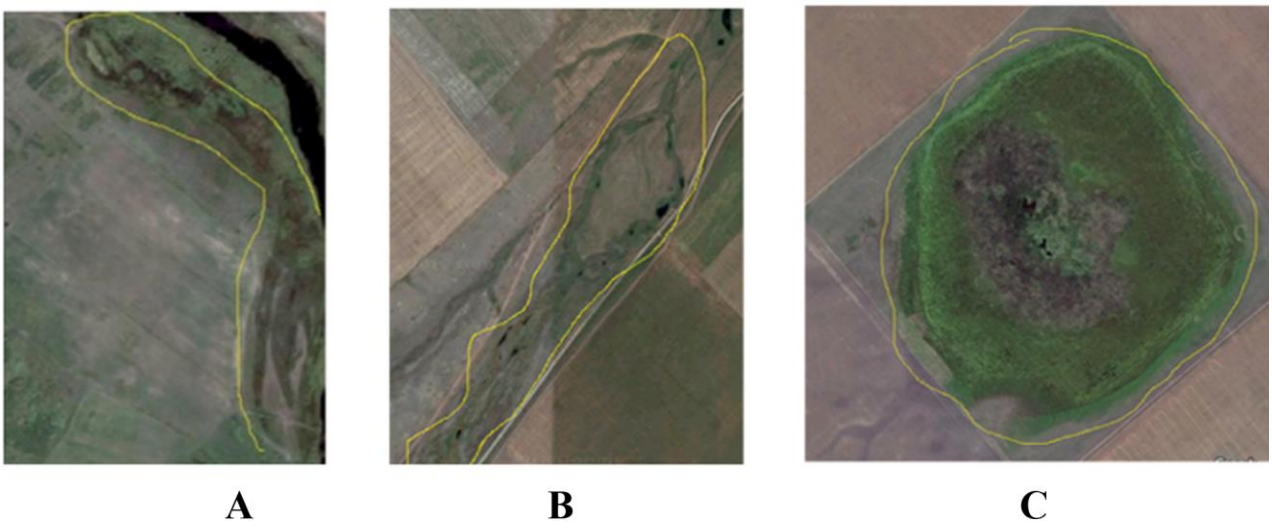

Figure 2. A – Wetland site № 2; **B –** Wetland site № 3; **C –** Wetland site №4

Dark-chestnut soils dominate the territory, most of which were ploughed during the development of virgin and fallow lands. The most widespread plant species include feather grass, wormwood, fescue, astragalus, burnet, and spurge. The area is characterized by the presence of floodplain bogs, which are formed due to spring floods and subsequent swamping, belonging to the lowland type. These bogs have a rich mineral content, supporting high species diversity.

In the course of expeditions, it was stated that this area is characterised by a floodplain type of bogs, which arise as a result of spring floods of the Aktastinka River and subsequent swamping of the resulting floodplains, belonging to the lowland type. As a rule, floodplain bogs have a rich mineral content, which determines a high species diversity of flora. The study of macrophytes in floodplain marshes is a multifaceted process involving a variety of research methods to fully understand their influence on the marsh ecosystem. Macrophytes, or aquatic plants, play a key role in the biodiversity and functioning of aquatic ecosystems, including wetlands. They provide shelter and food for various animal species and also perform water purification and bank stabilisation functions [28].

The macrophyte flora of the study area includes 105 species, classified into 65 genera and 38 families. Dicotyledonous plants dominate with 78 species, while monocotyledons account for

27 species. The ratio of dicotyledons to annuals is 1:2.9. The flora consists mainly of herbaceous polycarpic plants (75 species, 71.4% of the total). Herbaceous monocarpics are less common, making up 28.6% (30 species). The floristic spectrum includes five main subclasses: *Liliopsida, Ranunculidae, Caryophyllidae, Rosidae,* and *Asteridae* from the class *Magnoliopsida.*

In the course of the research, the flora of floodplain bogs of four study sites was described. The flora is understood as a system of the population of all plants growing in a given area. On average, each genus is represented by 2.3 species, and the species richness of families is characterized by an average of 9.5. The floristic spectrum of the floodplain flora of the neighbourhood of Aktasty village and the Atastinka River is represented by 5 main subclasses: *Liliopsida, Ranunculidae, Caryophyllludae, Rosidae*, and *Asteridae* from the class *Magnoliopsida.* Depending on the frequency of occurrence and abundance of individuals, the coastal macrophytes of the study sites were subdivided into six major groups according to O. Drude's scale, as shown in Table 1.

Table 1

The Most common families of Floodplain swamps of Aktasty village

№	Families	Number of genera	Number of species	Frequency of occurrence of the species according to O. Drude's scale
1.	*Equisetaceae*	1	1	Sol.
2.	*Nymphaeaceae*	1	2	Sp.
3.	*Typhaceae*	2	5	Cop.2
4.	*Caryophyllaceae*	2	3	Cop.2
5.	*Poaceae*	5	8	Soc.
6.	*Asteraceae*	5	7	Cop.
7.	*Fabaceae*	3	4	Cop.3
8.	*Limoniaceae*	1	2	Cop.3
9.	*Lamiaceae*	2	3	Cop.1
10.	*Chenopodiaceae*	2	2	Cop.3
11.	*Rosaceae*	3	7	Cop.1
12.	*Alliaceae*	2	3	Cop.3
13.	*Apiaceae*	2	4	Cop.3
14.	*Liliaceae*	3	4	Cop.2
15.	*Ranunculaceae*	2	3	Cop.2
16.	*Polygonaceae*	2	3	Cop.2
	Overall	37	61	

1 group *Cop.* 1 – abundant growth of representatives of families *Lamiaceae* and *Rosaceae.* 2 group *Cop.* 2 – abundant growth on the studied plots, there are many individuals of these families: *Typhaceae, Caryophyllaceae, Liliaceae, Ranunculaceae, Polygonaceae.* 3 group *Cop.* 3 – plants grow abundantly, but do not provide background *Fabaceae, Limoniaceae, Alliaceae, Apiaceae.* 4 group *Socails – (Soc.)* – plants interlocking with their above-ground parts, forming

a continuous background. This group is represented by 8 species of the *Poaceae* family. Growing in large quantities, they form continuous thickest several kilometres long; 5 groups – *Sol.* Plants are rare, single specimens. This group is characterised by a single representative of the family *Equisetacea*. 6 group *Sp.* – Rarely occur, scattered in small numbers – 2 species of the family *Nymphaeaceae.*

Autochthonous tendencies in the development of floodplain flora are practically not expressed, as evidenced by the absence of endemic species and the complete absence of polymorphic genera. From the data of macrophytes of floodplain bogs of the study area, the most frequently occurring species are represented by the following families *Poaceae* (8 species), *Asteraceae* (7 species), *Rosaceae* (7 species), *Typhaceae* (5 species), and three families are represented by four species such as *Fabaceae, Apiaceae,* and *Liliaceae.*

Analysis by life forms demonstrated that the studied flora is represented mainly by herbaceous polycarpics – 75 species, which is 71.4 % of the total identified flora. It is typical for temperate floras. Herbaceous monocarpics play a much smaller role in the composition of the floodplain macrophyte flora. Monocarpics are represented by 30 species, accounting for 28.6 %, respectively.

Of the total number of identified macrophytes of floodplain bogs, 8 species of vascular plants are the most common. Their location is marked with dots on the map shown in Figure 3. These species are often found and form a continuous background on the coastal territory of representatives of the *Poaceae* family, in particular, *Phragmites australis, Cyperaceae (Carex rostrata), and Typhaceae* Juss. *(Typha angustifólia), Ranunculaceae (Ranunculus repens L*), *Juncaginaceae (Triglochin maritimum L), Hydrocharitaceae* Juss. *(Stratiotes aloides L.;), Lentibulariaceae Utricularia vulgaris L.; Araceae – Lemna minor L.*

Figure 3. map-scheme of disturbution of floodplain macrophytes along the Aktastinka River and in wetlands: *1 - Phragmites australis; 2 - Carex rostrata; 3 - Typha angustifolia; 4- Ranunculus repens* L.*; 5 - Triglochin maritimum* L.*; 6 - Stratiotes aloides* L.*; 7 - Utricularia vulgaris* L.*; 8 - Lemna minor* L

Coastal macrophytes in the study sites were categorized into six groups according to O. Drude's scale, ranging from abundant species (Lamiaceae, Rosaceae, Typhaceae, Poaceae) to rare species (Equisetaceae, Nymphaeaceae). The most frequently occurring families include Poaceae (8 species), Asteraceae (7 species), Rosaceae (7 species), and Typhaceae (5 species). The most common macrophyte species were identified along the Aktastinka River and in wetlands, including Phragmites australis, Carex rostrata, Typha angustifolia, and Ranunculus repens.

Discussion

The study of macrophytes in floodplain marshes is a complex process requiring various research methods to understand their role in the ecosystem. Macrophytes play a critical role in biodiversity and ecosystem functionality, providing shelter and food for numerous species, contributing to water purification, and stabilizing riverbanks.

The high mineral content of floodplain bogs significantly influences the floristic composition, resulting in a diverse plant community. The predominance of herbaceous polycarpic plants is typical for temperate ecosystems, as these species are well adapted to periodic flooding and nutrient-rich soils. The absence of endemic species suggests a lack of significant autochthonous development, with the local flora influenced by broader regional patterns.

Along with the main bog macrophytes, other species were also observed, however, they were less frequent and formed small patches. The following species can be referred to them:

1. Inula britanica L. Stems 0.8-3.5 m tall, erect, glabrous, filamentous up to inflorescence, with hollow creeping rhizome. Leaves 05-0.25 cm wide, stiff, glaucous or pale green, glabrous, sometimes covered with sparse long hairs on the underside, sharply scabrous along the edges. Tongues are in the form of a border of short, dense hairs. Anthers 1,5-2,5 mm long. Frequency of occurrence: Copiosae

2. Ranunculus polyanthemos L. Stem erect, ribbed, simple. Leaves linear-lanceolate. The corms are 3-4(5) cm in diameter, in a loose shield. The wrapper is multi-rowed, with equally acute, bent leaflets. Uvular flowers are yellow. Tongues and teeth of tubular flowers are densely covered with golden glands on the outside. Frequency of occurrence Cop. 1

3. Typha angustifiolia Perennial rhizomatous plant. The plant reaches a height of 2.5 m. Leaves are linear at the base of the stem. Flowers are small, unisexual, with perianth. The inflorescence is a head-like, long-cylindrical cob. It usually grows in temperature climates, preferring moist areas. Frequency of occurrence: Cop.2

4. Butomus umbellatus L. The rhizome is creeping, thick. Flower stalks 40-100 cm tall, smooth, stout. Leaves long, linear, triangular at the base, 4 above, flat, 5-10 mm wide, erect, shorter than the stem. Inflorence is umbrella-shaped with numerous large pinkish flowers, on pedicels of unequal length. At the base of the umbrella, there are 3 covering triangular-lanceolate leaflates. Frequency of occurrence: Cop.2

5. Hydrocharis morsus-ranae L. Branched, unrooted, floating stems with long-petioled, rosette-like leaves floating on the water surface. Their lamina is rounded, broadly heart-shaped at the base, with two bracts. The perianth consists of three ovate pale green ovate sepals with dirty purple veins and three rounded white petals; Frequency of occurrence: Cop.3

6. *Equisetum fluviatile Riverside horsetail,* or Equisetum fluviatile, is a species of plant in the horsetail family. It typically grows in moist soils, including riverbanks, lakes, and marshes. Frequency of occurrence: Sol.

7. *Nymphaea candida J. Presl & C. Presl* (White water lily) White water lily, or Nymphaea candida, is a species of plant in the lily family. It usually grows in freshwater bodies, such as lakes and ponds, with deep and clear waters. Frequency of occurrence: Sp.

The categorization of macrophytes according to O. Drude's scale highlights the varying degrees of abundance and distribution of plant species. The dominance of Poaceae, Asteraceae, and Rosaceae families suggests their ecological significance in these floodplains. The presence of rare species such as Equisetum fluviatile and Nymphaea candida indicates specific microhabitats that support specialized flora.

Overall, the study confirms that the floodplain flora of the Aktastinka River is highly diverse, shaped by hydrological and soil conditions. The findings contribute to the broader understanding of floodplain ecosystems, emphasizing the importance of conservation efforts to maintain biodiversity and ecological balance in these wetland habitats

Conclusion

Thus, a comprehensive study of the species diversity of macrophytes growing along the Akastinka River and in the wetlands of Aktasty village was carried out. The species composition of vascular plant flora was revealed, and taxonomic and typological analyses were carried out. Four types of dominant plant communities were identified for the study area: reed, cattail-sedge, buttercup-onion, flowering rush-bladderwort, and other phytocenoses are represented by scattered patches.

Floristic composition of the local flora of Aktastinka village and coastal territory is characterised by rich species diversity of 105 species belonging to 65 genera and 38 families. The number of floodplain macrophytes totalled 15 taxa, 8 of which the main taxa forming continuous thickets during recent research: *Phrágmites austrális, Carex rostrata; Týpha angustifólia, Ranunculus repens* L., *Triglochin maritimum* L., *Stratiotes aloides* L., *Utricularia vulgaris* L., *Lemna minor* L. and 7 taxa that do not form continuous thickets and grow in the study area. Submerged macrophytes are represented by Nymphaea Candida J. Presl & C. Presl, *Lemna minor* L., Hydrocharis morsus-ranae L., *Týpha angustifólia.*

In **conclusion,** further research on macrophytes in floodplain bogs is necessary for a better understanding of their role and impact on the ecosystem. This will assist to ensure the sustainability and biodiversity of these unique natural complexes for future generations. Observations have shown that macrophytes growing on the coastal territory of the Aktastinka River for a long time lead to the replacement of dense soils with loose ones. Constant or periodic waterlogging of the soil surface is the main cause of land swamping.

Author Contributions

B.Z.G. – approval of the final version; **B.Z.G**. – responsibility for all aspects of the work; **B.Z.G**. – ensuring the integrity of all parts of the article; **D.A.D. –** significant contribution to the concept

and design of the study; **D.A.D., S.K.M.,** and **G.G.A** – data collection; **D.A.D., S.K.M.**, and **G.G.A –** analysis and interpretation; **M.Y.H.** – development of the research concept; M.Y.H. and **Zh.M.Zh.** – formulation of key objectives and goals; **A.G.M. and I.Z.I.** – preparation and editing of the text; **A.G.M.** and **I.Z.I.** – participation in the scientific design of the study; **S.K.N.** and **G.G.A** – conducting research; **A.N.Ye. –** composition of the article and design development; **Zh.M.Zh. –** formation of the idea.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this work.

Compliance with ethical standards

This article does not contain a description of studies performed by the authors involving people or using animals as objects.

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Ақмола облысының жайылмалы батпақтарының макрофиттері

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Аңдатпа. Бұл жұмыста Қазақстан Республикасының Аршалы ауданы аумағында орналасқан Ақтасты өзені жағалауы аумақтарының және Ақтасты ауылының сулы-батпақты жерлерінің жайылмалы батпақтарының макрофиттерін ұзақ мерзімді зерттеу нәтижелері келтірілген. Түгендеу барысында Ақтасты ауылының елді мекені мен жағалау аумағының флористикалық құрамы анықталды, оның құрамына 65 тұқымдас пен 38 тұқымдасқа жататын 105 түр кірді. Түрлердің алуан түрлілігімен қатар батпақты флора макрофиттерінің негізгі өкілдері 8 гидрофит – Phragmites australis, Carex rostrata арқылы анықталды; *Typha angustifolia, Ranunculus repens* L., *Triglochin maritimum* L., *Stratiotes aloides* L., *utricularia vulgaris* L., *lemna minor* L. және т.б. Зерттеу аймағының жергілікті флорасында өсімдіктер қауымдастығының төрт түрлі фитожүйелері құрылды олардын ішінде басым тұқымдастар: *Poaceae, Typhaceae, Cyperaceae, Ranunculaceae, Amaryllidaceae, Butomaceae, Lentibulariaceae.* Төрт түрлі учаскедегі макрофитті флораларды салыстырмалы талдау фитоценоздардың ұқсастығын көрсетті. Зерттелетін аймақтағы тамырлы өсімдіктердің таксономиялық құрамына 65 жалпы және 38 тұқымдастарға жататын 105 түр кіреді. Қосжарнақты өсімдіктер 78 түрден, біржарнақтылар 27 түрден тұрады. Қосжарнақтылардың біржылдықтарға қатынасы 1:2,9 құрады. Орташа алғанда, әрбір тұқым 2,3 түрден тұрады, тұқымдастардың түрлік қанықтылығы орташа көрсеткішпен сипатталады және 9,5 құрайды.

Түйін сөздер: макрофиттер, гидрофиттер, жағалаудағы өсімдіктер, түрлердің әртүрлілігі, жайылма батпақтар

Макрофиты пойменных болот Акмолинской области

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Аннотация. В данной статье представлены результаты многолетних исследований макрофитов пойменных болот прибрежных территорий реки Актастинка и заболоченных участков села Актасты, расположенного в Аршалынском районе Акмолинской области Республики Казахстан.

В ходе инвентаризации выявлен флористический состав окрестностей села Актастинка и прибрежной территории, составивший 105 видов, относящихся к 65 родам и 38 семействам. Наряду с видовым разнообразием определены основные представители макрофиты болотной флоры, представленные 8 гидрофитами – Phragmites australis, Carex rostrata; *Typha angustifolia, Ranunculus repens* L.*, Triglochin maritimum* L.*, Stratiotes aloides* L.*, Utricularia vulgaris* L.*, Lemna minor* L. В локальной флоре изучаемого участка установлены четыре типа растительных сообществ с доминированием представителей следующих семейств: *Poaceae, Typhaceae, Cyperaceae, Ranunculaceae, Amaryllidaceae, Butomaceae, Lentibulariaceae* протяженностью несколько километров. Сравнительный анализ флор макрофитов на четырех разных участках показал схожесть фитоценозов. Таксономический состав флоры сосудистых растений на исследуемом участке насчитывает 105 видов, относящихся к 65 родам и 38 семействам. Двудольные растения представлены 78 видами, однодольные 27 видами. Соотношение двудольных к однодольным составило 1:2,9. В среднем каждый род представлен 2,3 видами, видовая насыщенность семейств характеризуется средним показателем и составляет 9,5.

Ключевые слова: макрофиты, гидрофиты, прибрежные растения, видовое разнообразие, пойменные болота

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A novel non-paralytic botulinum neurotoxin type A for chronic pain management in animal models

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Abstract. Botulinum neurotoxin is one of the most potent biological toxins known, capable of causing severe paralysis by blocking neurotransmitter release. The use of botulinum neurotoxin (BoNT) has grown beyond its traditional application for muscle overactivity disorders, now being explored for the treatment of various chronic pain conditions such as chronic migraine (CM) and painful diabetic peripheral neuropathy (PDPN). This article aimed to assess the therapeutic potential of a newly engineered botulinum neurotoxin molecule, el-iBoNT, in animal models of CM and PDPN pain. Utilizing the innovative SpyCatcher–SpyTag protein conjugation method, we successfully produced functional botulinum neurotoxin molecules with significantly reduced paralytic effects compared to the native toxin. In both CM and PDPN models, a single administration of el-iBoNT resulted in substantial pain relief, alleviating both mechanical and thermal hypersensitivity. The findings demonstrate that el-iBoNT holds promise as an effective therapeutic agent for managing chronic pain in these conditions. Additionally, the reduced paralytic activity of el-iBoNT suggests a safer profile compared to traditional BoNT therapies. Overall, this research supports the potential of el-iBoNT as a novel treatment option for chronic pain, offering a promising alternative to existing pain management strategies, particularly those that rely on opioids, which often carry the risk of dependency and severe side effects.

Keywords: non-paralytic botulinum neurotoxin, el-iBoNT, chronic migraine, painful diabetic peripheral neuropathy, mechanical sensitivity, temperature sensitivity, rat grimace scale

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Introduction

Botulinum neurotoxin type A (BoNT/A) is the longest-acting serotype due to its unique mechanism of action, involving the cleavage of the SNAP25 target protein (9 amino acids). This results in prolonged inhibition (approximately 6 months) of SNARE complex function in presynaptic nerve terminals [1-3]. In neuromuscular terminals, BoNT/A blocks acetylcholine exocytosis, leading to muscle relaxation. This property has found applications in cosmetic procedures and the treatment of conditions such as strabismus, blepharospasm, and hemifacial spasm [4-6]. When acting on sensory nerve endings associated with pain, BoNT/A exhibits additional mechanisms of action. These include inhibiting the release of nociceptive neurotransmitters at peripheral terminals [7-8], modulating the expression of ion channels and pain receptors [9], and exerting effects within the central nervous system [10-12].

Despite its therapeutic potential, the paralytic effects of native BoNT/A limit its broader application. To address this issue, several laboratories have developed modified, non-paralytic botulinum molecules using advanced technologies. These include SNARE-stapling constructs such as Binary Toxin (BiTox), Binary Toxin/AA (BiTox/AA), Tetanus Toxin (TetBot), Substance P Toxin (SP-Bot), Dermorphine Toxin (Derm-Bot), and isopeptide-bonded molecules like iBoNT and elongated iBoNT (el-iBoNT), developed using the SpyCatcher–SpyTag system [13]. This study aims to evaluate the analgesic potential of the newly engineered el-iBoNT molecule as a non-paralytic treatment for chronic pain in animal models.

Materials and research methods

Botulinum Preparations

The experiments utilized native incobotulinumtoxinA, Xeomin® (Merz Pharma GmbH & Co. KGaA, Germany), and a novel non-paralytic botulinum toxin, el-iBoNT, prepared at the University of Sheffield (Sheffield, UK). To produce el-iBoNT, represented as (1) Light chain-Translocation domain–syntaxin 1A–SpyCatcher – (2) SpyTag–Heavy Chain, two recombinant proteins – (1) and (2) – were expressed in the BL21(DE3) Escherichia coli strain. Competent cell transformation was performed via heat shock using the pGEX-KG vector. Recombinant proteins fused with glutathione S-transferase (GST) were purified on glutathione-Sepharose beads (GE Healthcare, USA), followed by thrombin cleavage. Further purification was achieved using affinity chromatography on a Superdex 200 10/200 GL column (GE Healthcare, USA). The protein yield was approximately 200 μg per liter of bacterial culture. The el-iBoNT complex was assembled by mixing LC-Td–syx–SpyCatcher and SpyTag–HC for 2 hours at 4°C in Buffer A. Purified proteins were aliquoted and stored at -80°C for subsequent experiments.

Experimental Animals

The study subjects were sexually mature male laboratory rats bred and raised under the controlled conditions of the academic and research laboratory facility at Al-Farabi Kazakh National University (Almaty, Kazakhstan).

Chronic Pain Models

A nitroglycerin (NTG)-induced chronic migraine model was established via five intraperitoneal NTG injections (10 mg/kg) administered every two days over nine days (Figure 1A) [14]. A streptozotocin (STZ)-induced model of diabetic peripheral neuropathic pain was created using a single intraperitoneal STZ injection (45 mg/kg) (Figure 1B) [15].

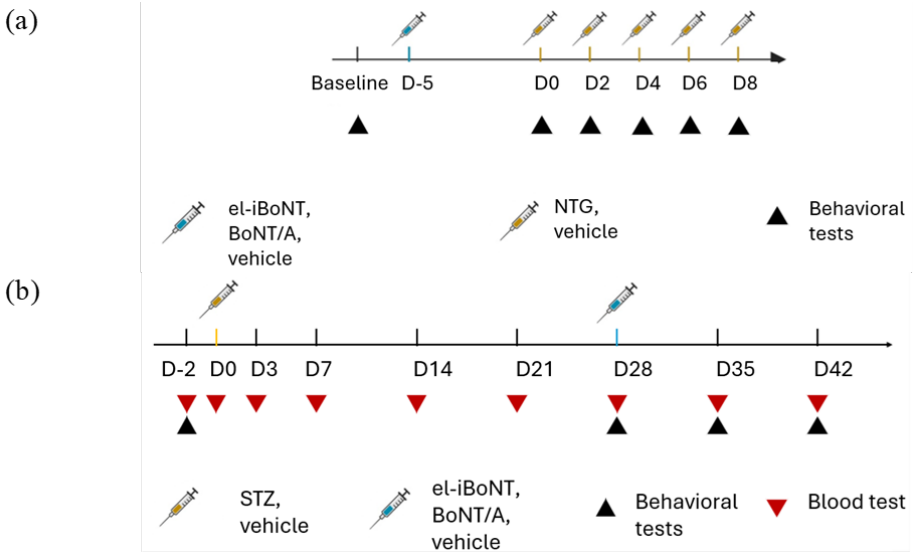


Figure 1. Timeline of behavioral testing: **(a)** CM model; **(b)** DPNP model

Behavioral Tests

The Rat Grimace Scale (RGS) was used to quantify pain by assessing specific facial features across four distinct action units: orbital tightening, nose/cheek flattening, ear changes, and whisker changes. Each action unit was scored on a 3-point scale (0 = no change, 1 = moderate change, 2 = obvious change) [16]. The von Frey Test involved placing rats in plexiglass chambers on an elevated grid and allowing them to acclimatize. Following acclimatization, the plantar surface of the paw was stimulated with a von Frey filament (BioSeb, France). The pain threshold was defined as the force (in grams) at which the rat withdrew its paw. The Hargreaves Test involved placing rats in plexiglass chambers on an elevated platform. After acclimatization, the plantar surface of the paw was stimulated with a heat beam (Hargreaves Apparatus, Ugo Basile, Italy). The pain threshold was defined as the time (in seconds) before the paw was withdrawn.

Injections

In the NTG model, prophylaxis was performed via bilateral periorbital injections of 2.5 U Xeomin®/10 ng el-iBoNT seven days before the first induced migraine episode. In the STZ model, pain control was administered on day 28 following STZ injection. This involved plantar injections of 5 U Xeomin®/20 ng el-iBoNT. Additionally, el-iBoNT (50 ng, 100 ng, and 150 ng) was injected into the gastrocnemius muscle to visually assess the in vivo paralytic activity of the molecules.

Statistical Analysis

Statistical analyses were performed using Prism 10.1.1 (GraphPad Software, La Jolla, CA, USA) and IBM SPSS Statistics for Windows, Version 29.0.1.0 (Armonk, NY, USA).

Results

The non-paralytic botulinum neurotoxin el-iBoNT differs from the native toxin in its structural characteristics (Figure 2). The length and mass of el-iBoNT are 22.8 nm and 182,255 Da, respectively, which are higher than those of the native toxin, which measures 12.5 nm and 149,425 Da.

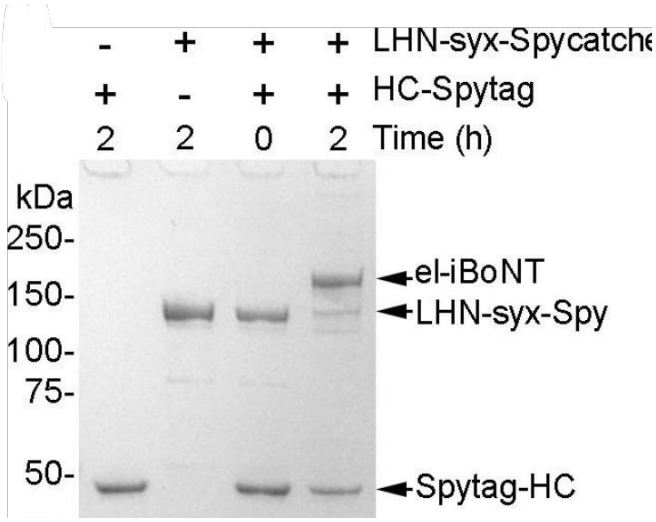


Figure 2. SDS-PAGE of el-iBoNT

It was demonstrated that when up to 150 ng of the new preparation was injected into the right gastrocnemius muscle of the rat, no motor dysfunction symptoms were observed, and the rats maintained normal mobility (Figure 3). However, rats exposed to 20 U of BoNT/A showed a characteristic response: the toes of the ipsilateral paw were clenched, the leg was extended, and the rat could not bear its weight. The animals exhibited jumping movements and had difficulty balancing while standing on their hind legs.

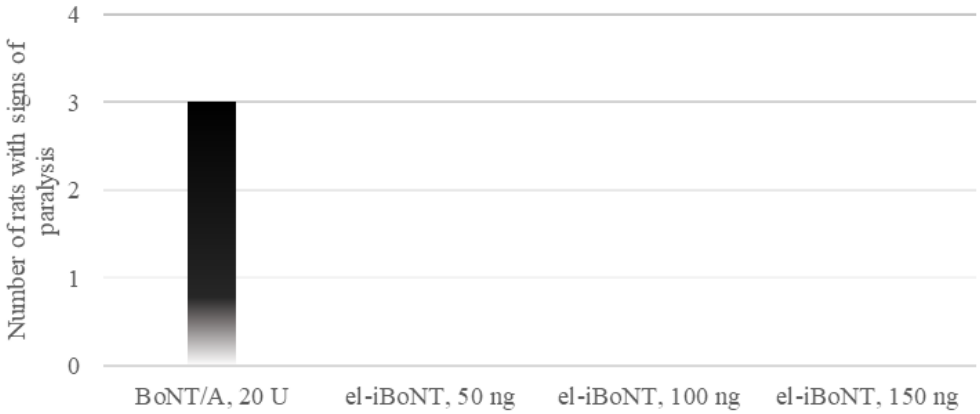
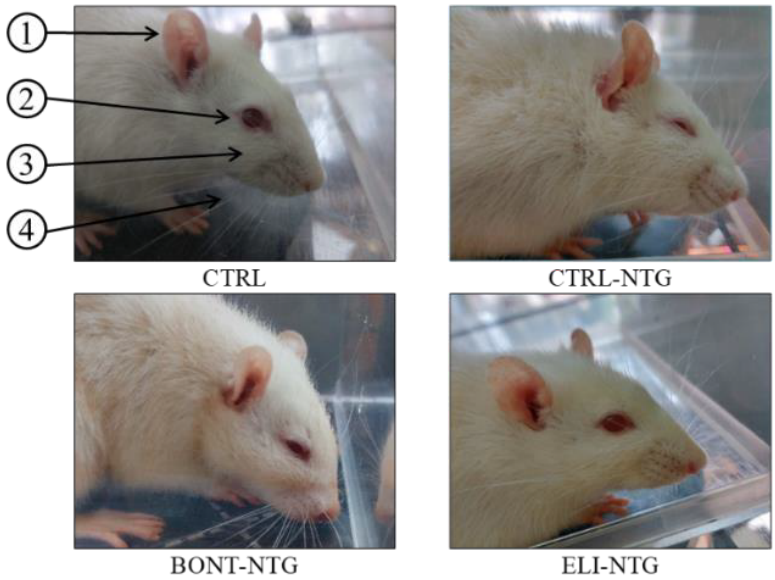


Figure 3. Histogram showing the number of rats affected by botulinum neurotoxin injections (n = 3)

The CM model involved 24 animals, which were divided into 4 groups (6 animals in each group) depending on the administered substances: 1. CTRL (n=6): saline + saline; 2. CTRL-NTG (n=6): saline + nitroglycerin (NTG), 10 mg/kg; 3. BONT-NTG (n=6): botulinum toxin type A (BoNT/A), 5 U + NTG, 10 mg/kg; 4. ELI-NTG (n=6): modified botulinum toxin (el-iBoNT), 20 ng + NTG, 10 mg/kg.

A total of 144 images were obtained, which were evaluated by two independent researchers using the rat grimace scale. Representative photographs are shown in Figure 4. The overall Cronbach's alpha coefficient was 0.81, with the highest score for orbital narrowing (0.93).

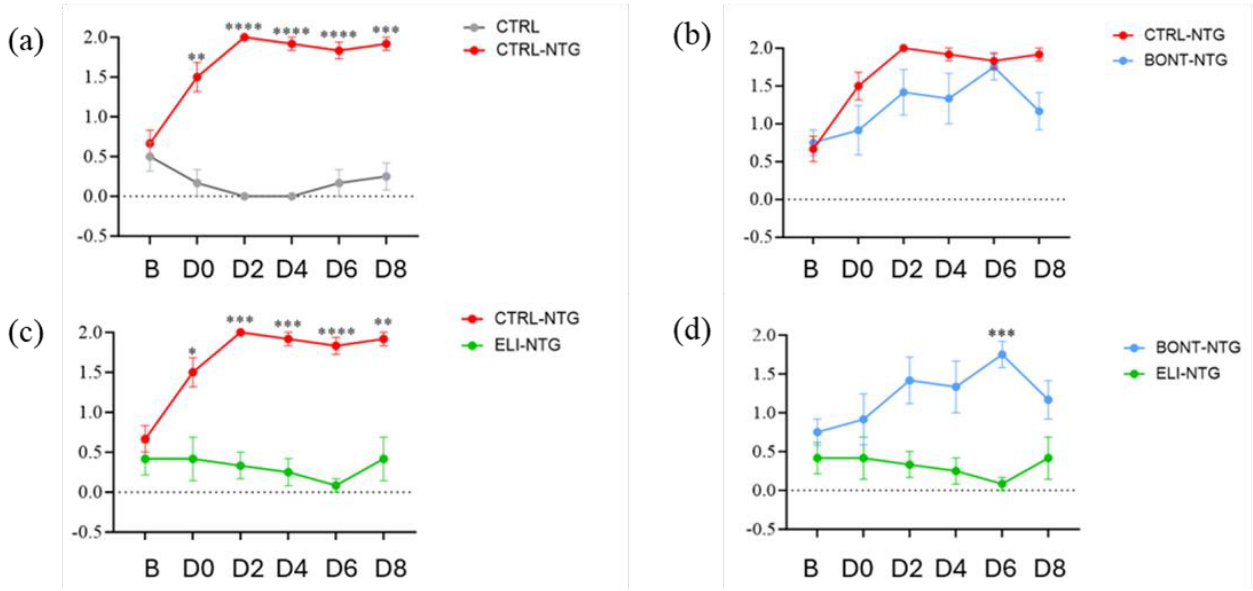


Note: 1 – ear change, 2 – orbital narrowing, 3 – smoothing of nose/cheeks, 4 – vibrissae change

Figure 4. Representative images of rat grimaces

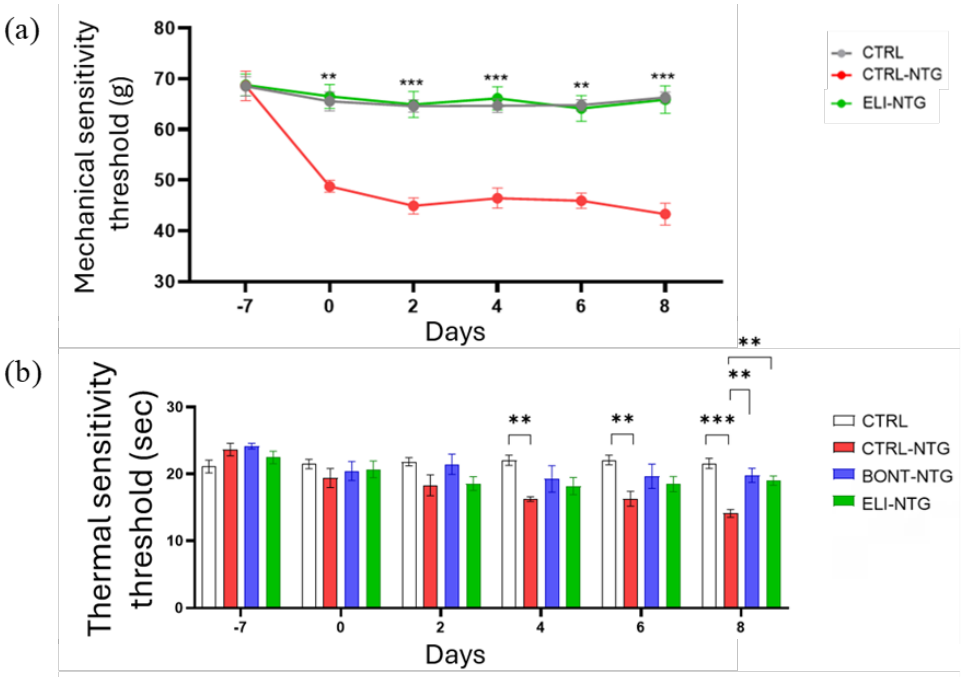
Groups CTRL and CTRL-NTG showed significant differences in the intergroup comparison of grimace scale (GS) and orbital narrowing (ON) on Days 0-8. No significant differences were found between the groups CTRL-NTG and BONT-NTG throughout the experiment. Significant differences between groups CTRL-NTG and ELI-NTG were observed in most cases (Days 2-8 for GS, Days 0-8 for ON). Animals in the ELI-NTG group also showed statistically significantly lower scores compared to BONT-NTG (Days 2, 6 for GS, Day 6 for ON; Figure 5).

On the days of nitroglycerin injection (10 mg/kg, i.p.), the animals developed mechanical hypersensitivity measured as a decrease in the mechanical threshold on the hind paws. Extracranial injections of el-iBoNT (20 ng) five days prior to CM induction maintained the mechanical threshold at levels comparable to native animals (Figure 6A). On the fourth, sixth, and eighth days of nitroglycerin injections, animals developed thermal hypersensitivity, measured by a decrease in the latency period in response to heating the medial surface of the hind paw. Extracranial injections of BoNT/A and el-iBoNT maintained the thermal threshold at levels comparable to native animals only on Day 8 (Figure 6B).



Note: B – baseline, D0 – session 1, D2 – session 2, D4 – session 3, D6 – session 4, D8 – session 5; CTRL (n=6), CTRL-NTG (n=6), BONT-NTG (n=6), ELI-NTG (n=6); asterisks indicate intragroup differences. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 5. Intergroup comparison of orbital narrowing: **(a)** CTRL vs CTRL-NTG; **(b)** CTRL-NTG vs BONT-NTG; **(c)** CTRL-NTG vs ELI-NTG; **(d)** BONT-NTG vs ELI-NTG

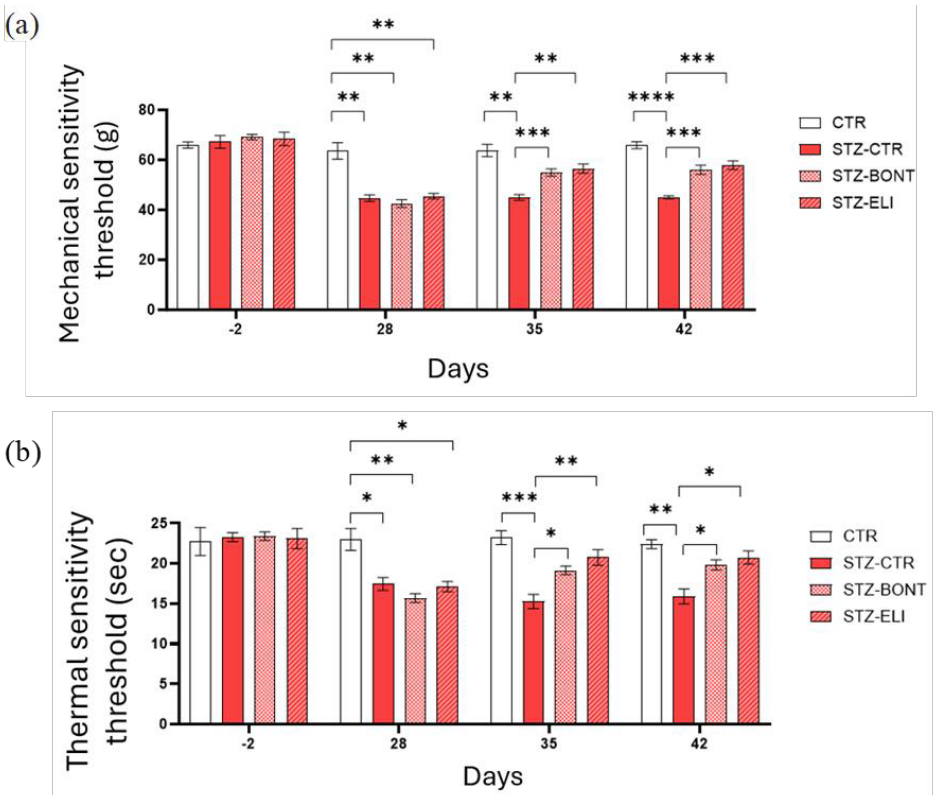


Note: -7 – seven days before CM induction, 0, 2, 4, 6, 8 – days of CM induction; CTRL (n=6), CTRL-NTG (n=6), BONT-NTG (n=6), ELI-NTG (n=6); asterisks indicate intragroup differences compared to baseline levels. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 6. Changes in **(a)** mechanical and **(b)** thermal hypersensitivity NTG model

The PDPN model involved 26 animals, which were divided into 4 groups as follows: 1. CTR (n=6): saline + saline; 2. STZ-CTR (n=6): streptozotocin (STZ), 45 mg/kg + saline; 3. STZ-BONT (n=7): STZ, 45 mg/kg + BoNT/A, 5 U; 4. STZ-ELI (n=7): STZ, 45 mg/kg + el-iBoNT, 20 ng.

The average blood glucose level in the STZ group was above 410 mg/dl (non-fasting) throughout the experiment. Other signs of diabetes were observed in this group, including weight loss, polydipsia, polyuria, and polyphagia (data not provided). In these animals, a single unilateral injection of BoNT/A (5 U) or el-iBoNT (20 ng) into the hind paw significantly reduced both mechanical (Figure 7A) and thermal hypersensitivity (Figure 7B).



Note: -2 – 7 days before the induction of diabetes, 0, 3, 7, 14, 21, 28, 25, 42 – days after STZ injection; CTR (n=6), STZ-CTR (n=6), STZ-BONT (n=7), STZ-ELI (n=7); asterisk indicates within-group differences compared to baseline levels. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 7. Changes in **(a)** mechanical and **(b)** thermal hypersensitivity in the PDPN model

Discussion

Attempts to develop non-paralytic variants of botulinum neurotoxin have been made previously. The protein stapling technique was used to generate BiTox [17, 18], BiTox/AA [19], TetBot [20], SP-Bot [21, 22], and Derm-Bot [21, 23]. However, the stapling method required the assembly of three polypeptides, and pain relief was only achieved at high nanogram doses, which

raises concerns about potential immune responses upon repeated administration. Moreover, the non-covalent nature of the stapling process means that dissociation of the components cannot be ruled out [24].

Unlike these stapled molecules, el-iBoNT was designed using a different approach: isopeptide bonding. Notably, the novel elongated botulinum neurotoxin has only been investigated in one study to date, where it was shown to treat nerve injury pain without causing muscle paralysis [24]. This technique allows for the spontaneous covalent linkage of the two botulinum components upon mixing, ensuring molecular stability. The results of our study show that el-iBoNT effectively reduces mechanical and thermal hypersensitivity in animal models of chronic migraine and painful diabetic peripheral neuropathy.

One of the most notable advantages of el-iBoNT is the lower required dosage for effective pain relief. For example, while BiTox required hundreds of nanograms for analgesic effects [18], el-iBoNT was effective at 20 ng, suggesting improved potency. Moreover, SP-Bot and Derm-Bot [21] target specific neuronal populations (NK1R and μ-opioid receptors, respectively), whereas el-iBoNT retains a broader mechanism of action, making it applicable to multiple pain conditions.

Another key advantage of el-iBoNT is its significantly reduced paralytic activity compared to both native BoNT/A and other engineered variants. While traditional BoNT/A blocks neurotransmission at both sensory and motor nerve terminals, leading to pain relief but also muscle paralysis, el-iBoNT was specifically designed to avoid neuromuscular toxicity while maintaining analgesic effects. This distinction is particularly evident when comparing el-iBoNT with iBoNT. Electromyographic analysis has shown that animals injected with iBoNT exhibited significant motor deficits, whereas el-iBoNT-treated animals retained normal motor function. Furthermore, immunohistochemical studies demonstrated that iBoNT caused stronger cleavage of SNAP25 in neuromuscular junctions, whereas el-iBoNT had minimal activity at these sites, explaining its lack of paralytic effects [24].

While the full mechanisms underlying the analgesic effects of el-iBoNT remain to be elucidated, recent findings suggest that it may share common pathways with BoNT/A. The analgesic effects of BoNT/A are believed to involve inhibition of neurotransmitter release (CGRP, Substance P, glutamate), modulation of nociceptive ion channels (TRPA1, TRPV1, P2X3), and retrograde transport to central pain-processing areas [25, 26]. It was demonstrated that el-iBoNT shares at least some of the key features of BoNT/A action. Firstly, Leese et al. confirmed that el-iBoNT cleaves SNAP25 in TRPV1-positive sensory fibers, further supporting its role in modulating nociceptive transmission via ion channel regulation. Additionally, el-iBoNT has been shown to suppress microglial activation in the dorsal horn, which may contribute to its central antinociceptive effects [24].

Taken together, these findings confirm that el-iBoNT retains the key analgesic mechanisms of BoNT/A while offering a potentially improved safety profile due to its reduced paralytic activity. Further studies are warranted to explore its long-term effects on neurotransmitter release, ion channel expression, and microglial function to fully define its therapeutic potential. In addition, clinical trials will be necessary to confirm the safety and efficacy of el-iBoNT in human patients.

While we demonstrated the absence of paralysis, additional studies are required to evaluate potential off-target effects.

Conclusion

The new findings presented here can be divided into several key conclusions. First, it was demonstrated that the SpyCatcher–SpyTag protein conjugation approach allows for the creation of a functional botulinum neurotoxin. This method represents a safe approach to producing botulinum molecules for therapeutic use. Second, it was shown that the el-iBoNT molecule exhibits significantly lower paralytic activity compared to the native toxin, highlighting the importance of structure in the action of molecules derived from BoNT/A. Third, el-iBoNT effectively alleviates pain induced by systemic administration of nitroglycerin in the NTG-induced chronic migraine model. Fourth, the effectiveness of el-iBoNT in pain therapy was demonstrated in the STZ-induced diabetic peripheral neuropathy pain model. Together, our study suggests that the engineered el-iBoNT molecule could become a new therapeutic agent for individuals suffering from chronic migraine and diabetic peripheral neuropathy. This is crucial given the currently limited therapeutic options for treating chronic pain, which are often ineffective, cause intolerable side effects, and contribute to the opioid crisis.

Author Contributions

B.D., A.K. – concept and supervision of the work; **A.Z. –** conducting the experiments; **A.Z., B.D., A.K. –** discussion of the research results; **A.Z. –** writing the text; **A.Z., B.D.,** and **A.K.** – editing the text of the article. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Compliance with ethical standards

The experiments were conducted with the approval of the local ethics committee of Al-Farabi Kazakh National University (Almaty, Kazakhstan), protocol №IRB-377 dated 24.02.2022. All procedures performed in studies involving animals complied with the ethical standards of the institution where the studies were conducted and the approved legal acts of the Republic of Kazakhstan and international organizations.

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Жануарлардың ауырсыну үлгілеріндегі созылмалы ауырсынуды емдеуге арналған А типті жаңа ботулиннің паралич емес молекулалары

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Аңдатпа. Ботулиндік нейротоксин – нейротрансмиттерлердің бөлінуін бөгей отырып, ауыр салдануды тудыратын ең күшті биологиялық токсиндердің бірі. Ботулиндік нейротоксин (BoNT) дәстүрлі түрде бұлшықет гиперактивтілігін емдеуде қолданылып келген, алайда қазіргі уақытта ол әртүрлі созылмалы ауырсыну жағдайларын, оның ішінде созылмалы мигрень (CM) және ауыр диабеттік перифериялық нейропатия (PDPN) сияқты ауруларды емдеу үшін қолданылып келеді. Бұл зерттеу жаңа инжинирингтелген ботулиндік нейротоксин молекуласы, el-iBoNT, оның созылмалы мигрень мен ауыр диабеттік перифериялық нейропатия моделдеріндегі терапевтік әлеуетін бағалауды мақсат етті. SpyCatcher–SpyTag ақуызды байланыстырғыш әдісін пайдалана отырып, біз функционалды ботулиндік нейротоксин молекулаларын сәтті өндірдік, олар бастапқы токсинге қарағанда айтарлықтай төмен паралитикалық белсенділікке ие.

СМ және PDPN модельдерінде el-iBoNT бір рет енгізілгеннен кейін ауырсынуды айтарлықтай жеңілдетіп, механикалық және термиялық гиперсезімталдықты төмендетті. Бұл зерттеу нәтижелері el-iBoNT-тің созылмалы ауырсынуды басқару үшін тиімді терапевтік агент ретінде қолданылатынын көрсетеді. Сонымен қатар, el-iBoNT-тің төмен паралитикалық белсенділігі оның дәстүрлі BoNT терапияларына қарағанда қауіпсіз екендігін көрсетеді. Жалпы, бұл жұмыс el-iBoNT-ті жаңа емдеу нұсқасы ретінде дамытудың мүмкіндігін қолдайды, бұл созылмалы ауырсынуды жеңілдетуге және дәстүрлі ауырсынуды емдеу стратегияларына, оның ішінде опиоидтарға тәуелділікті азайтуға мүмкіндік береді.

Түйін сөздер: паралитикалық емес ботулиндік нейротоксин, el-iBoNT, созылмалы мигрень, ауыр диабеттік перифериялық нейропатия, механикалық сезімталдық, температуралық сезімталдық, егеуқұйрықтардың бет-әлпеті шкаласы

Новый непаралитический ботулинический нейротоксин типа А для лечения хронической боли в животных моделях боли

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Аннотация. Ботулинический нейротоксин – один из самых мощных биологических токсинов, способный вызывать тяжелый паралич, блокируя высвобождение нейротрансмиттеров. Использование ботулинического нейротоксина (BoNT) расширяется за пределы традиционного применения для лечения расстройств гиперсокращения мышц и активно исследуется для лечения различных хронических болевых состояний, таких, как хроническая мигрень (ХМ) и болевая диабетическая периферическая нейропатия (БДПН). Целью данного исследования было оценить терапевтический потенциал новой молекулы ботулинического нейротоксина, el-iBoNT, в моделях болевого синдрома при ХМ и БДПН. Используя инновационный метод конъюгации белков SpyCatcher–SpyTag, мы успешно создали функциональную молекулу ботулинического нейротоксина с существенно сниженной паралитической активностью по сравнению с нативным токсином. В обеих моделях после однократного введения el-iBoNT наблюдалось значительное облегчение боли, что проявилось в снижении как механической, так и температурной гиперчувствительности. Эти результаты демонстрируют, что el-iBoNT представляет собой эффективный терапевтический агент для лечения хронической боли при ХМ и БДПН. Кроме того, сниженная паралитическая активность el-iBoNT предполагает его более безопасный профиль по сравнению с традиционными препаратами BoNT. В целом данное исследование подтверждает потенциал el-iBoNT как новой терапии хронической боли, предлагая перспективную альтернативу существующим стратегиям лечения боли, особенно тем, которые основаны на применении опиоидов, часто сопряженных с риском зависимости и серьезных побочных эффектов.

Ключевые слова: непаралитический ботулинический нейротоксин, el-iBoNT, хроническая мигрень, болевая диабетическая периферическая нейропатия, механическая чувствительность, температурная чувствительность, шкала гримас крыс

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Мониторинг распространенности и антибиотикорезистентности *Acinetobacter baumannii* в многопрофильном стационаре Астаны

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Аннотация. *Acinetobacter baumannii* – одна из самых опасных внутрибольничных инфекций, приводящих к летальности больных с ослабленным иммунитетом, особенно в отделениях реанимации и интенсивной терапии. Многие штаммы устойчивы к карбапенемам – «антибиотикам последней линии». Это делает лечение больных крайне сложным. В связи с этим была изучена частота выделения и уровень резистентности штаммов *A. baumannii* у пациентов детского кардиохирургического отделения (ДКХО) и отделения анестезиологии, реанимации и интенсивной терапии (ОАРИТ) за период с 2015 по 2022 годы. Проведен ретроспективный анализ результатов бактериологического посева различных биоматериалов на выделение патогенной флоры, взятых от взрослых пациентов, госпитализированных в ОАРИТ, и пациентов ДКХО за 8 лет с определением антибиотикочувствительности. Было выделено 8696 клинических изолятов, среди которых *A. baumannii* составил 629 случаев, преимущественно из ОАРИТ (203) и ДКХО (426). Идентификация проводилась на микробиологическом анализаторе «Vitek 2 – Compact» (bioMerieux, Marcy l'Etoile, France). Антимикробная активность была исследована по методу минимальных ингибирующих концентраций («Vitek 2 – Compact»). Анализ распределения изолятов демонстрирует значительную разницу в частоте обнаружения A. baumannii в отделениях ОАРИТ и ДКХО: 13,5% в ОАРИТ против 5,8% в ДКХО. Анализ устойчивости к антибиотикам выявил рост резистентности ко всем используемым антибиотикам, особенно в ОАРИТ, где резистентность к левофлоксацину была 93,3%, меропенему-92,8%, гентамицину-90,6%, ципрофлоксацину-89,7% и имипенему-86,1%. В ДКХО также зафиксирован рост резистентности, но в пределах 27%. Таким образом, сравнительный анализ частоты выделения A. baumannii в отделениях ОАРИТ был выше, чем в ДКХО.

Ключевые слова: внутрибольничные инфекции, *Acinetobacter baumannii,* антибиотикорезистентность, карбапенемы, колистин, множественная устойчивость

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Введение

В последние десятилетия *Acinetobacter baumannii* привлекает внимание ученых из-за его множественной лекарственной устойчивости [1, 2]. В списке ВОЗ он отнесен к категории критического приоритета из-за резистентности к карбапенемам и цефалоспоринам третьего поколения [3-5]. Это приводит к увеличению длительности госпитализации и смертности пациентов, создавая серьезные трудности для врачей и работников здравоохранения.

Штаммы *A. baumannii* представляют особую угрозу в отделениях реанимации, интенсивной терапии и кардиохирургии [1-6]. Повышение устойчивости к карбапенемам, которые используются как антибиотики последней линии, усугубляет проблему [7-11]. Рост числа штаммов с множественной лекарственной устойчивостью увеличивает частоту таких нозокомиальных инфекций, как вентилятор-ассоциированная пневмония, катетер-ассоциированные инфекции мочевыводящих путей и инфекции центрального кровотока, что повышает затраты на лечение и риск летального исхода [12-14].

A. baumannii – один из наиболее распространенных инфекционных агентов, отличающийся устойчивостью к дезинфицирующим средствам, ультрафиолетовому излучению, высушиванию и антибиотикам различных классов [15]. За последние 13 лет в структуре возбудителей гнойных ран наблюдается увеличение доли *K. pneumoniae* и *A. baumannii*. Бактерия способна длительное время сохраняться в больничной среде и на медицинском оборудовании, способствуя распространению инфекций [16].

Инфекции, вызванные*A. baumannii*, особенно опасны для пациентов с ослабленным иммунитетом, включая новорожденных и реанимационных больных. Бактерия способна длительно сохраняться в организме и на больничных поверхностях, обладает высокой антибиотикорезистентностью и вирулентностью, что увеличивает заболеваемость и смертность [17, 18]. Она также играет ключевую роль в развитии гнойно-септических осложнений у пациентов с ожогами [19, 20]. После трансплантации легких заражение *A. baumannii* снижает выживаемость пациентов [21].

A. baumannii не только развивает устойчивость к антибиотикам, но и образует биопленки на медицинских устройствах, таких, как венозные катетеры и эндотрахеальные трубки, что значительно осложняет лечение. Распространение биопленкообразующих штаммов среди множественно устойчивых популяций *A. baumannii* представляет серьезную клиническую проблему [22]. Факторы, способствующие его распространению, включают длительную госпитализацию, наличие сопутствующих заболеваний, сложность лечения в отделениях интенсивной терапии и широкое применение противомикробных препаратов.

Неонатальный сепсис остается одной из ведущих причин младенческой смертности, особенно в странах с низким уровнем дохода (СНД) [23]. Появление множественно устойчивых к антибиотикам патогенов, включая *Acinetobacter spp.,* значительно осложняет лечение и повышает риск неблагоприятных исходов [23]. Исследования подтверждают роль *A. baumannii* в развитии тяжелых инфекций, включая остеомиелит и инфекции кожи и мягких тканей, особенно в условиях стационаров [24]. Кроме того, *A. baumannii* часто выявляется у пациентов с послеоперационными инфекциями центральной

нервной системы, демонстрируя устойчивость к большинству протестированных антибиотиков [25]. Также его ассоциируют с орально-дентальными заболеваниями, особенно у пациентов с ослабленным иммунитетом и возрастными заболеваниями [26]. Устойчивость *A. baumannii* к нескольким классам антибиотиков остается серьезной проблемой [27]. В связи с этим целью данного исследования является анализ частоты выделения и уровня резистентности *A. baumannii* у пациентов детской кардиохирургии и ОАРИТ многопрофильного стационара Астаны за 2015–2022 годы.

Материалы и методы исследования

Объекты исследования

Исследование проведено на основании анализа биоматериалов, полученных от взрослых пациентов, госпитализированных в отделение анестезиологии, реанимации и интенсивной терапии (ОАРИТ), а также от пациентов детского кардиохирургического отделения (ДКХО) за период с 2015 по 2022 годы. Основное внимание уделено выделению и идентификации патогенной микрофлоры с последующим определением чувствительности к антимикробным препаратам. В фокусе исследования находился клинически значимый вид грамотрицательных бактерий *Acinetobacter baumannii.*

Отбор биологических материалов

Биологический материал включал образцы из респираторного тракта (мазки из зева, мокрота, бронхоальвеолярный лаваж, плевральная жидкость, содержимое катетеров трахеостомы, интубационные трубки), мочевыводящих путей (образцы мочи и содержимое мочевых катетеров), а также материалы, связанные с раневыми и катетер-ассоциированными инфекциями (содержимое дренажей, мазки из ран, центральных венозных катетеров, санационных и аспирационных катетеров, мазки из пролежней). Весь клинический материал собирался и транспортировался в микробиологическую лабораторию согласно методическим рекомендациям.

Микробиологический анализ и идентификация изолятов

Количественный анализ исследуемого материала проводили с использованием различных питательных сред, включая кровяной агар, среду Эндо, желточно-солевой агар, *Candida* агар, агар Калины, шоколадный агар и хромогенные агары. Посевы инкубировали при 37°C в течение 24 часов. В соответствии с методическими рекомендациями идентификацию изолятов осуществляли на основании морфологических свойств, окраски по Грамму, оксидазного и каталазного тестов, а также тестов на плазмокоагулазу, желчный тест и индолообразование. Заключительную идентификацию чистых культур проводили с использованием микробиологического анализатора «Vitek 2 – Compact» (bioMérieux, Marcy l'Étoile, France). Чувствительность выделенных штаммов к антимикробным препаратам, включая меропенем, имипенем, амикацин, гентамицин, тобрамицин, ципрофлоксацин и левофлоксацин, определяли методом минимальных ингибирующих концентраций (МИК) с применением «Vitek 2 – Compact».

Статистический анализ данных

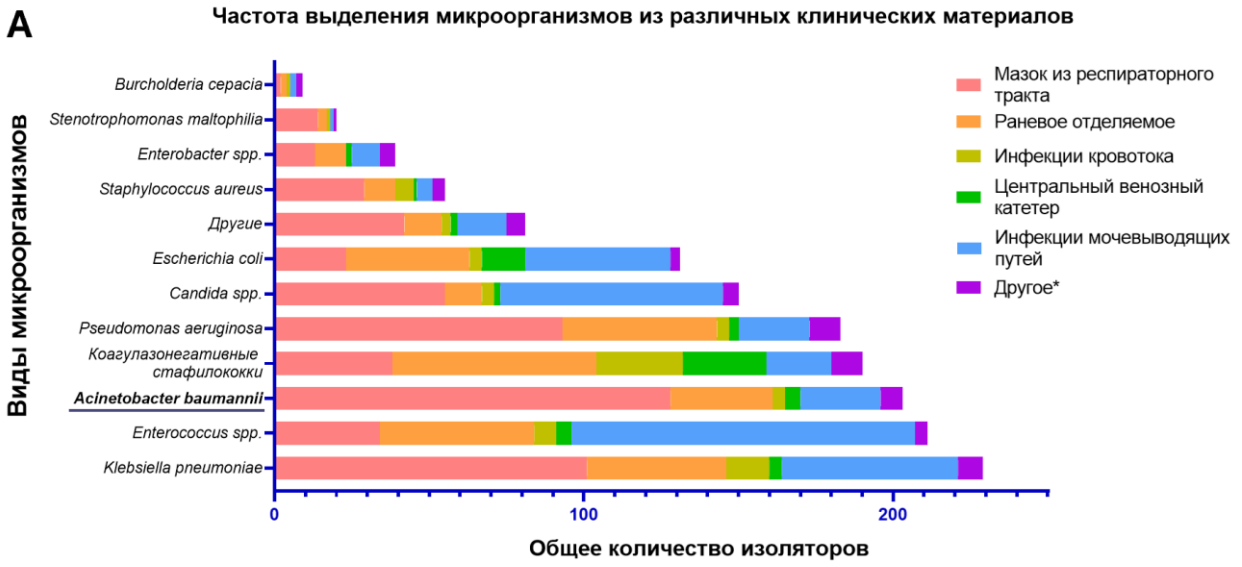
Статистическую обработку полученных данных проводили по χ^2 (хи-квадрат) и t-тексту Стюдента с помощью GraphPad prism 8.0.1. Различия средних значений считались по уровеню доверительного интервала P <0,05. Особенности статистического анализа и формирования данных для обработки описаны снизу диаграммы в примечаниях.

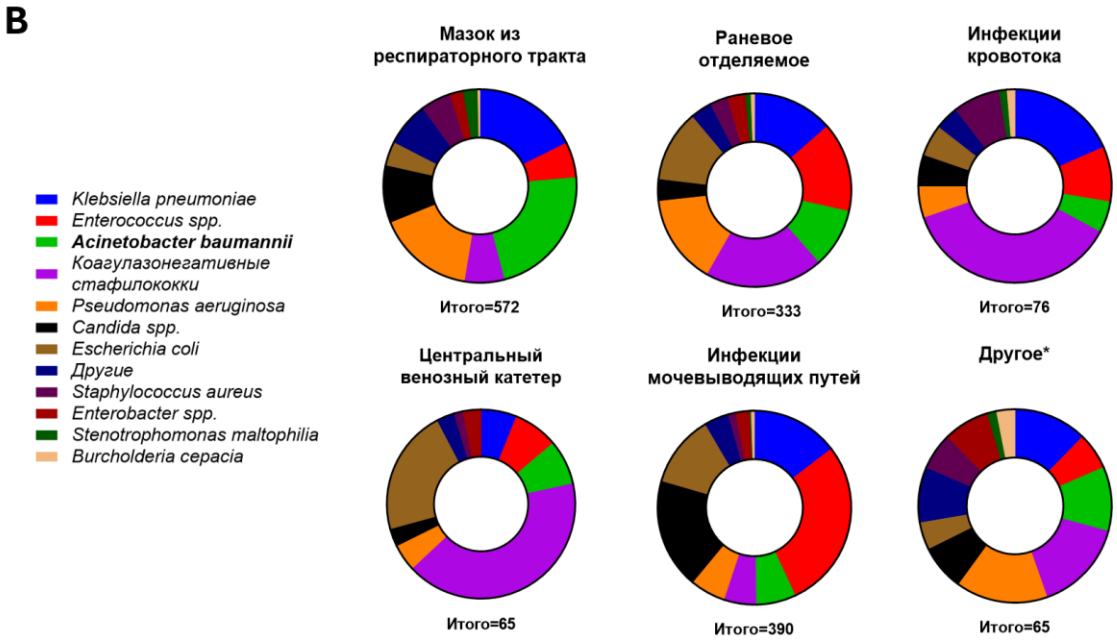
Результаты и обсуждение

Частота высева различных микроорганизмов из ОАРИТ

В ходе исследования были изучены частота высева различных микроорганизмов из отделения анестезиологии, реанимации и интенсивной терапии (ОАРИТ) из различных клинических материалов у пациентов за 2015-2022 гг. (Рисунок 1). Всего в ходе исследования высева различных микроорганизмов из ОАРИ были проанализированы 1501 посев. Наибольшая частота выделения наблюдалась для *Klebsiella pneumoniae* (15,3%) и *Acinetobacter baumannii* (13,5%), преимущественно из мазков респираторного тракта, раневого отделяемого и *Enterococcus spp.* (14,1%) из инфекций мочевыводящих путей (Рисунок 1А). В меньшей степени были представлены *Burcholderia cepacia* (0,6%) и *Stenotrophomonas maltophilia* (1,3%).

Также определено, что соотношение высева зависимости от источника клинического материала (Рисунок 1В). В мазках респираторного тракта преобладали *A. baumannii* (22,4%), *K. pneumoniae* (17,7%) и Pseudomonas aeruginosa (16,3%). В раневом отделяемом наиболее часто выделялись коагулазонегативные стафилококки (CoNS, 19,8%), *K. pneumoniae* (15%) и *Enterococcus* spp. (15%). В инфекциях кровотока наибольшее клиническое значение имели *CoNS* (36,8%) и K. pneumoniae (18,4%). В центральном венозном катетере доминировали CoNS (41,5%) и *Escherichia coli* (21,5%), в мочевыводящих путях *Enterococcus spp*. (28,5%), *Candida spp.* (18,5%) и *K. pneumoniae* (14,6%). В группе «кончик санационного катетера, кончик аспирационного катетера, жидкость из плевральной полости», объединённой в Другие*, чаще выделялись CoNS (15,4%), *P. aeruginosa* (15,4%) и *K. pneumoniae* (12,3%).



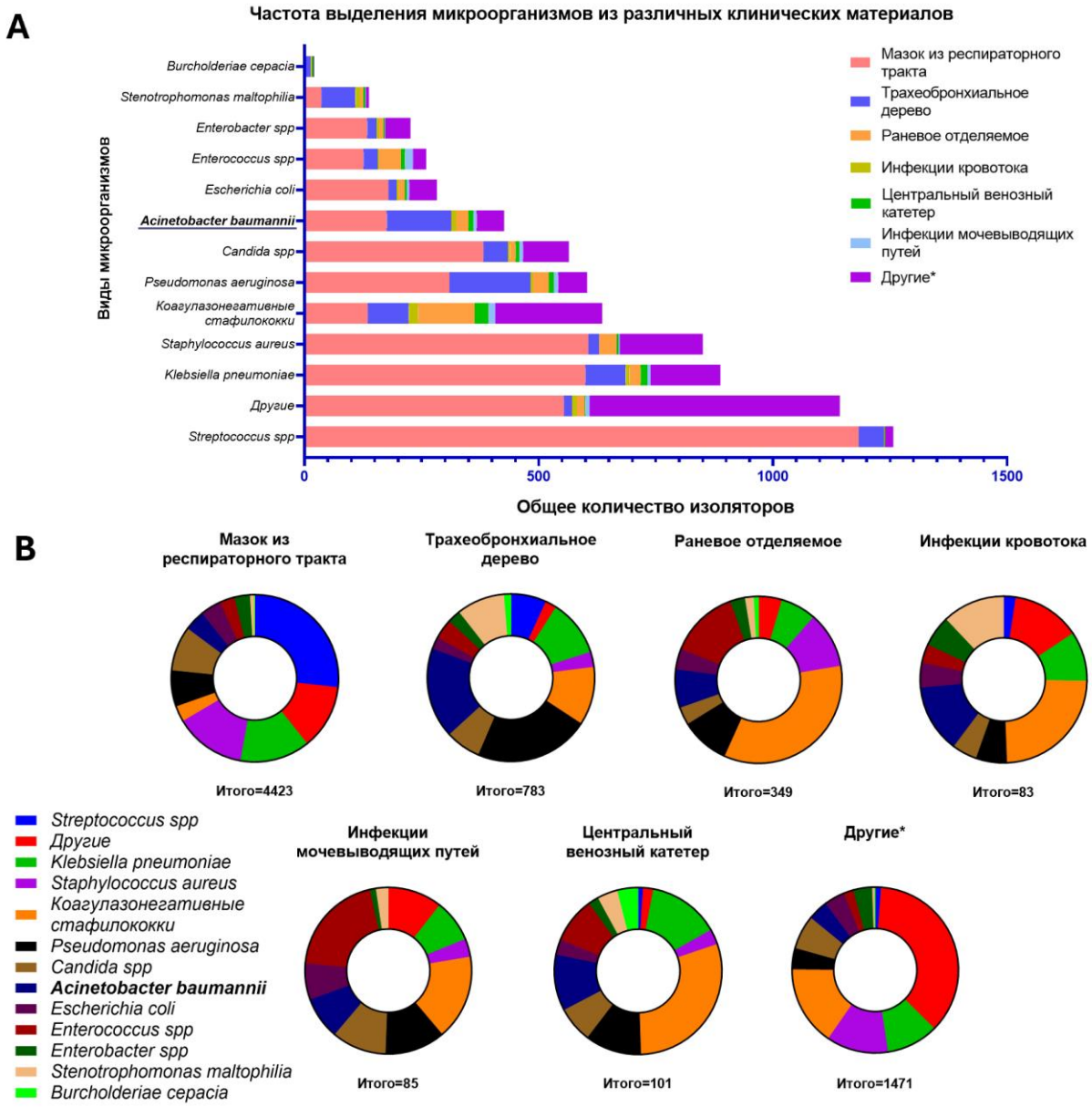


Примечание: *кончик санационного катетера, кончик аспирационного катетера, жидкость из плевральной полости. Статический анализ по χ^2 показал P <0,0001. Данные формировали соединением значений выделения *Enterobacter spp., Stenotrophomonas maltophilia, Burcholderia* cepacia к группе микроорганизмов Другие.

Рисунок 1. Частота выделения микроорганизмов из различных клинических материалов пациентов из отделения анестезиологии, реанимации и интенсивной терапии (ОАРИТ). **(А)** общее количество изолятов для каждого вида микроорганизмов с учетом их происхождения; **(В)** долевое соотношение отдельных видов микроорганизмов в зависимости от источника

Частота высева различных микроорганизмов из ДКХО

При изучении высева различных микроорганизмов из детского кардиохирургического отделения (ДКХО) из различных клинических материалов у пациентов за 2015-2022 гг. была добавлена дополнительная группа «трахеобронхиальное дерево», которая включала изоляты из содержимого катетеров трахеостомы и интубационных трубок. Это было связано с тем, что за данный период из ДКХО было выделено и идентифицировано 7295 штаммов (Рисунок 2). Дополнительно была выделена дополнительная группа при определении видов микроорганизмов Staphylococcus spp.



Примечание: *кончик санационного катетера, кончик аспирационного катетера, жидкость из плевральной полости. Статический анализ по χ^2 показал P <0,0001. Данные формировали соединением значений выделения *Staphylococcus aureus* и коагулазонегативные стафилококки к *Streptococcus spp,* и соединением значений выделения *Burcholderiae cepacia* группе микроорганизмов Другие.

Рисунок 2. Частота выделения микроорганизмов из различных клинических материалов пациентов из детского кардиохирургического отделения (ДКХО). **(A)** общее количество изолятов для каждого вида микроорганизмов с учетом их происхождения; **(B)** долевое соотношение отдельных видов микроорганизмов в зависимости от источника

Можно отметить, что картина высевов из ДКХО (Рисунок 2А) сильно отличалась от высевов пациентов в ОАРИТ (Рисунок 1А). Так как наибольшая частота выделения наблюдалась для *Staphylococcus spp.* (17%), другие микроорганизмы (16%), видовая специфичность которых не показана в данной статье (Другие) и *K. pneumoniae* (12,1%). *B. cepacia* (0,3%) и *S. maltophilia* (1,9%) также были представлены в меньшей степени, как и в данных ОАРИТ.

Долевое соотношение отдельных видов микроорганизмов в зависимости от источника клинического материала также показала отличимые результаты. *Streptococcus spp.* (26,7%), *Staphylococcus aureus* (13,7%) и *K. pneumoniae* (13,6%) преобладали в мазках респираторного тракта. В то время как в трахеобронхиальном дереве чаще высевали *P. aeruginosa* (15,4%) и *A. baumannii* (17,5%). В раневом отделяемом наиболее часто выделялись CoNS (34,4%) и *Enterococcus spp.* (13,5%), как и из клинического материала ОАРИТ. В то время как в инфекциях кровотока наибольшее клиническое значение имели CoNS (8%) и E. coli (13,3%) и *A. baumannii* (13,3%).

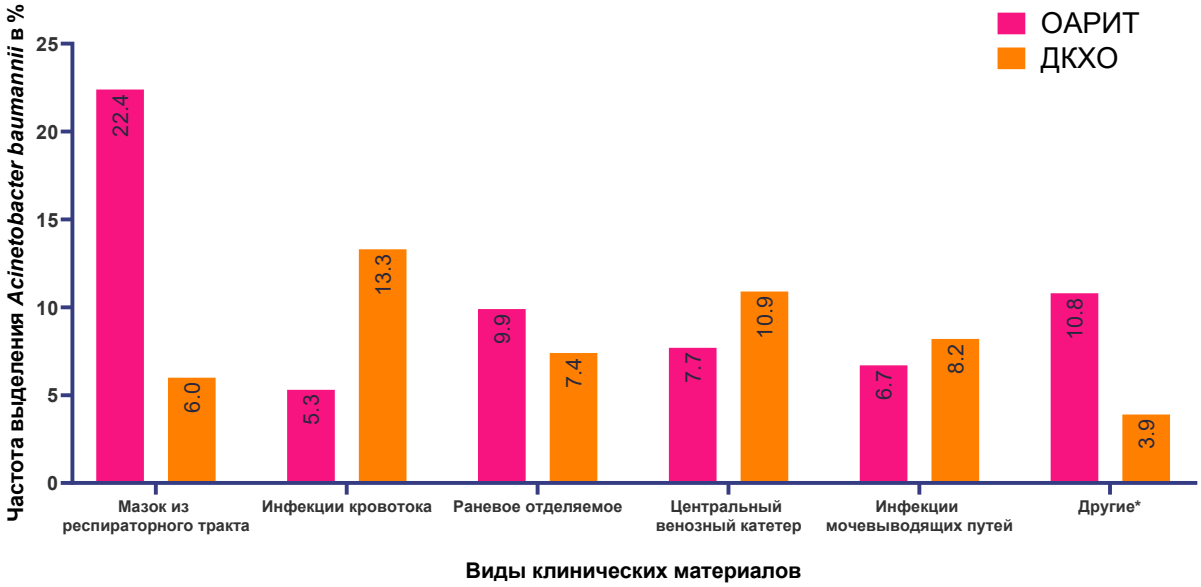
При высевах клинических материалов в центральном венозном катетере, полученных из ДКХО, доминировали CoNS (29,7%) и *K. pneumoniae* (13,9%). В мочевыводящих путях наиболее часто выделяли *Enterococcus spp.* (20%) и CoNS (16,5%). В группе Другие* преобладали другие микроорганизмы (36,3%) и CoNS (15,6%).

Оценка частоты выделения A. baumannii

A. baumannii было выделено из исследуемых материалов в ОАРИТ 13,5% (n-203) случаях и в ДКХО 5,8% (n-426) случаях. Для оценки частоты выделения *A. baumannii* из различных клинических материалов у пациентов ОАРИТ и ДКХО мы использовали процентные значения высевов по отношению к другим видам микроорганизмов. Данные с мазков респираторного тракта и трахеобронхиального дерева были объединены для наглядности исследования. Полученные данные проиллюстрированы на рисунке 3.

Согласно полученным данным, высеваемость *A. baumannii* из респираторных органов пациентов ОАРИТ составила 22%, а в ДКХО всего 6% (176), что демонстрирует значительную разницу в показателях; из центральной венозной крови в ОАРИТ обнаруживается 7,7%, в то время в ДКХО 10,9%; из раневого отделяемого в ОАРИТ – 9,9%, в ДКХО 7,4% – имеется несущественная разница в показателях. При сравнении результатов высеваемости A. baumannii при инфекциях кровотока и мочеполовых путей оказалось больше в ДКХО, которая составляет 13,3% и 8,2%, соответственно, в ОАРИТ эти показатели составляли 5,3% и 6,7%. В Другие*, где объединены значения из кончик санационного катетера, кончик аспирационного катетера, жидкость из плевральной полости, у пациентов ОАРИТ было идентифицировано *A. baumannii* в 10,8% и у пациентов ДКХО 3,9%.

Полученные данные подтверждают высокую распространенность *A. baumannii* в ОАРИТ, что может быть связано с тяжелым состоянием пациентов, длительной ИВЛ и инвазивными манипуляциями [28]. В ДКХО же *A. baumannii* чаще встречается при инфекциях кровотока и мочевыводящих путей, что требует дальнейшего изучения механизмов внутрибольничной передачи и разработки стратегий инфекционного контроля.



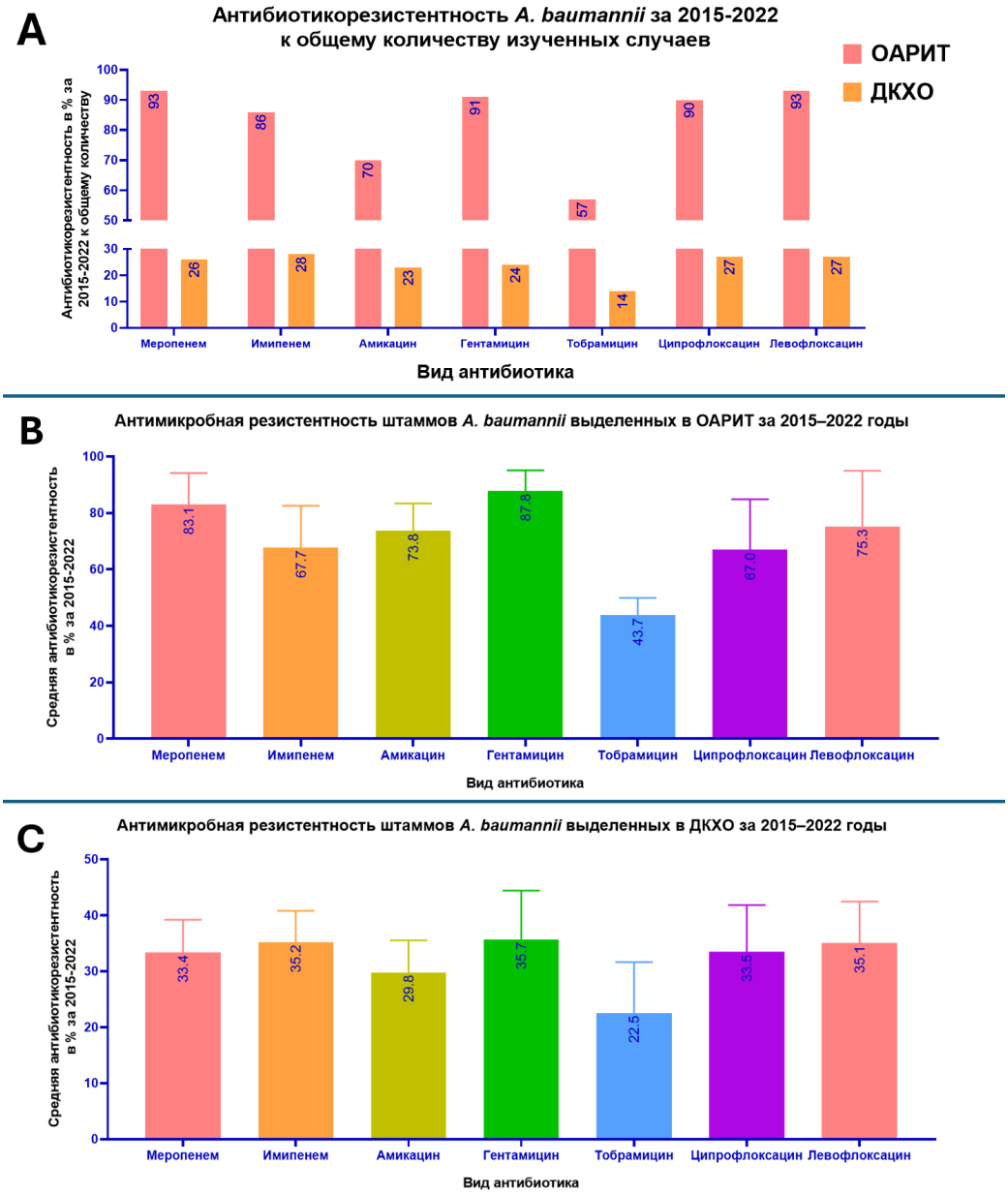
Примечание: *кончик санационного катетера, кончик аспирационного катетера, жидкость из плевральной полости.

Рисунок 3. Частота высеваемости *A. baumannii* из различных клинических материалов у пациентов в отделение анестезиологии, реанимации и интенсивной терапии (ОАРИТ) и от пациентов детского кардиохирургического отделения (ДКХО) за 2015-2022

Изучение антибиотикорезистентности A. baumannii

Как известно, штаммы *A. baumannii* являются особенно трудными патогенами в плане лечения, потому что широкий уровень антимикробной резистентности данного инфекционного агента вызывает ряд проблем при проведении антимикробной терапии на практике. За исследуемый период (2015-2022) было проведено 853 исследования на антибиотикорезистентность штаммов *A. baumannii* (Рисунок 4). Анализы проводились как общей группировкой, так и по отельным видам антибиотиков.

В ходе высевов антибиотикорезистентность штаммов *A. baumannii* определено, что в ОАРИТ было значимо выше, чем в ДКХО (P <0,0001). В ОАРИТ к левофлоксацину 93%, меропенему 93%, гентамицину 91%, ципрофлоксацину 90% и имипенему 86%, что согласуется с данными некоторых зарубежных авторов [27,29]. Тем не менее за исследуемый период не обнаружено резистентности к колистину, хотя некоторые авторы отмечают рост устойчивости *A. baumannii* к колистину [30,31]. Более того, отмечается ко всем протестированным антибиотикам ДКХО к имипенему 28%, левофлоксацину 27%, ципрофлоксацину 27%, меропенему 26,3% и гентамицину 24%.



Примечание: статистическая достоверность антибиотикорезистентность по отношению ОАРИТ к ДКХО составила P <0,0001 по t-тесту (Рисунок А). Статический анализ по χ^2 показал уровень достоверности P = 0,03 для рисунка В и P <0,0001 для рисунка С. В рисунке В и С графики построены по знамени средних и стандартной ошибки.

Рисунок 4. Анализ антибиотикорезистентности *A. baumannii* за исследуемый период 2015-2022 из материалов, полученных у пациентов отделения анестезиологии, реанимации и интенсивной терапии (ОАРИТ) и от пациентов детского кардиохирургического отделения (ДКХО). **(А)** к общему количеству изученных случав; **(В)** средняя антибиотикорезистентность выделенных с ОАРИТ и (С) ДКХО

Таким образом, анализ результатов антибиотикограмм не позволяет рекомендовать данные препараты как для комбинированной терапии, так и в монотерапии. По результатам нашего исследования наиболее эффективным в отношении штаммов *A. baumannii* являлся тобрамицин (в ОАРИТ 57% и ДКХО 14% резистентных изолятов) и амикацин (в ОАРИТ 70% и ДКХО 23%). Данные по антибиотикорезистентности, анализированные на средние значения выявленных случаев в год в ОАРИТ и ДКХО также показаны на рисунках 4B и 4C, соответственно.

Обсуждение

Анализ частоты выделения микроорганизмов у пациентов ОАРИТ и ДКХО за период 2015-2022 годы позволяет глубже понять динамику внутрибольничных инфекций и устойчивости к антибиотикам. Согласно полученным результатам, *Acinetobacter baumannii* занимает значительное место среди выделенных микроорганизмов, однако уступает ряду других условно-патогенных бактерий, что может свидетельствовать о влиянии терапевтических стратегий, направленных на контроль его распространения.

Изучение этиологического спектра патогенов ОАРИТ выявило широкое распространение и динамику увеличения частоты обнаружения штаммов A. baumannii. *A. baumannii* составляли 629 ед., из них 203 штамма выделено в ОАРИТ, 426 из ДКХО. Высеваемость *A. baumannii* в большинстве случаев наблюдалась из таких клинических материалов, как содержимое респираторного тракта, трахеобронхиального дерева, крови, ЦВК, раневого отделяемого и т. д.

Одним из ключевых аспектов исследования является оценка динамики резистентности *A. baumannii* к антибиотикам в разные годы, поскольку изменения в частоте его высеваемости могут быть обусловлены как эволюционными процессами, так и изменениями в схемах антимикробной терапии [1-8]. Выделенные штаммы характеризовались высоким уровнем устойчивости к основным группам антимикробных препаратов, что отражает сложившуюся тенденцию «проблемных» патогенов в настоящее время [18-21]. Это подтверждает необходимость комплексного подхода к исследованию антибиотикорезистентности, включающего молекулярно-генетический анализ, изучение механизмов горизонтального переноса генов и биохимические исследования адаптивных стратегий бактерий [24-28].

В клиническом аспекте данные исследования позволяют улучшить тактику эмпирической терапии, определить наиболее уязвимые группы пациентов и предотвратить дальнейшее распространение резистентных штаммов [29-30]. Кроме того, понимание механизмов устойчивости и динамики распространения *A. baumannii* в больничной среде имеет важное значение для эпидемиологического мониторинга и разработки профилактических мер, направленных на снижение заболеваемости и смертности, связанных с инфекциями, вызванными данным патогеном.

Заключение

Результаты данного исследования подчеркивают значимость *A. baumannii* как одного из ведущих патогенов в условиях интенсивной терапии, особенно в ОАРИТ и ДКХО. Существенные различия в частоте выделения этого микроорганизма между отделениями указывают на особенности эпидемиологической ситуации и факторы, способствующие его распространению. В ОАРИТ *A. baumannii* преимущественно ассоциирован с инфекциями респираторного тракта и раневых поверхностей, тогда как в ДКХО он чаще выявляется при инфекциях трахеобронхиального дерева, кровотока и мочевыводящих путей. Эти данные свидетельствуют о необходимости дифференцированного подхода к профилактике и лечению инфекций, вызванных этим патогеном, с учетом специфики каждого отделения.

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Соблюдение этических норм

Все процедуры, выполненные в исследованиях с участием людей, соответствуют этическим стандартам национального комитета по исследовательской этике и Хельсинкской декларации 1964 года и ее последующим изменениям или сопоставимым нормам этики.

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Астана қаласындағы көпсалалы ауруханада *Acinetobacter baumannii* таралуы мен антибиотиктерге төзімділігін бақылау

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Аңдатпа. *Acinetobacter baumannii* иммунитеті төмен науқастарда, әсіресе реанимация бөлімшелерінде өлімге әкелетін ең қауіпті ауруханаішілік инфекциялардың бірі болып табылады. Көптеген штамдар карбапенемдерге, «соңғы шараның антибиотиктеріне» төзімді. Бұл науқастарды емдеуді өте қиындатады. Осыған байланысты 2015-2022 жылдар аралығында балалар кардиохирургиялық бөлімшесінің (БКХБ) және анестезиология, реанимация және қарқынды терапия бөлімшелерінің (АРҚТБ) пациенттеріндегі *A. baumannii* штамдарының оқшаулану жиілігі мен төзімділік деңгейі зерттелді. АРҚТБ және БКХБ жатқызылған ересек пациенттерден және 8 жыл бойы алынған патогенді флораны бөліп алу үшін әртүрлі биоматериалдардың бактериологиялық нәтижелеріне антибиотикке сезімталдықты анықтау арқылы ретроспективті талдау жүргізілді. Барлығы 8696 клиникалық изоляттар оқшауланды, оның ішінде *A. baumannii* 629 жағдайды құрады, негізінен АРҚТБ (203) және БКХБ (426). Сәйкестендіру «Vitek 2 – Compact» микробиологиялық анализаторында (bioMerieux, Marcy I'Etoile, Франция) жүргізілді. Микробқа қарсы белсенділік ең аз ингибиторлық концентрация әдісімен («Vitek 2 – Compact») сыналды. Изоляттардың таралуын талдау АРҚТБ және БКХБ бөлімде A. baumannii анықтау жиілігінде айтарлықтай айырмашылықты көрсетеді: АРҚТБ – 13,5%, БКХБ – 5,8%. Антибиотиктерге төзімділік талдауы барлық қолданылатын антибиотиктерге төзімділіктің жоғарылауын анықтады, әсіресе АРҚТБ-де левофлоксацинге төзімділік 93,3%, меропенемге – 92,8%, гентамицинге – 90,6%, ципрофлоксацин – 89,7% және имипенемге – 86,1% құрады. БКХБ-де төзімділіктің жоғарылауы да тіркелді, бірақ 27% шегінде. Осылайша, *A. baumannii*-ның АРҚТБ-да оқшаулану жиілігінің салыстырмалы талдауы БКХБ қарағанда жоғары болды.

Түйін сөздер: ауруханаішілік инфекциялар, *Acinetobacter baumannii,* антибиотиктерге төзімділік, карбапенемдер, колистин, көп резистенттілік

Monitoring the prevalence and antibiotic resistance of *Acinetobacter baumannii* in a multidisciplinary hospital in Astana

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Abstract. *Acinetobacter baumannii* is one of the most dangerous hospital-acquired infections, leading to death in patients with weakened immune systems, especially in intensive care units. Many strains resist carbapenems, the "antibiotics of last resort," making treatment extremely difficult. In this regard, the frequency of isolation and the level of resistance of *A. baumannii* strains in patients of the pediatric cardiac surgery department (PCSD) and the department of anesthesiology, resuscitation, and intensive care

(DARIC) for the period 2015 to 2022 were studied. A retrospective analysis of the results of bacteriological culture of various biomaterials for the isolation of pathogenic flora taken from adult patients hospitalized in DARIC and PCSD patients over 8 years, with determination of antibiotic sensitivity was carried out. A total of 8696 clinical isolates were recovered, of which *A. baumannii* accounted for 629 cases, primarily from DARIC (203) and PCSD (426). Identification was carried out on a microbiological analyzer "Vitek 2 - Compact" (bioMerieux, Marcy I'Etoile, France). Antimicrobial activity was studied by the method of minimum inhibitory concentrations ("Vitek 2 - Compact"). Isolate distribution analysis shows a significant difference in the detection rate of *A. baumannii* in DARIC and PCSD: 13.5% in DARIC versus 5.8% in PCSD. Antibiotic resistance analysis revealed an increase in resistance to all antibiotics used, particularly in DARIC, where resistance to levofloxacin was 93.3%, meropenem 92.8%, gentamicin 90.6%, ciprofloxacin 89.7%, and imipenem 86.1%. PCSD also recorded an increase in resistance, but within 27%. Thus, the comparative analysis of the frequency of isolation of A. baumannii in DARIC units was higher than in PCSD. An increase in resistance to all tested antibiotics was noted in DARIC and PCSD units, except for colistin.

Keywords: hospital-acquired infections, *Acinetobacter baumannii*, antibiotic resistance, carba-penems, colistin, multiple resistance

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Модификация геномов растений методами генетической инженерии и геномного редактирования: доставка экзогенных ДНК

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Аннотация: Рассматриваются данные о развитии и совершенствовании методов доставки фрагментов экзогенных ДНК в геномы различных видов растений в постгеномную эру биологии. Приведены особенности доставки экзогенных ДНК в зависимости от этапа культивирования растительных клеток в условиях in vitro. Сделан акцент на развитии методов, обеспечивающих доставку кассет экспрессии в геномы растительных клеток *in planta,* исключающих дезинтеграцию растительных тканей до клеточных культур с последующим восстановлением растений-трансформантов, т.е. минуя стадию *in vitro.* Приведены данные об успешности применения метода *floral dip* как для модельных растений *Arabidopsis thaliana,* так и для некоторых других видов, являющихся представителями шести семейств высших растений. Приведены данные о востребованности метода *floral dip* в связи с возрастающим интересом к модификации растений методами геномного редактирования.
Ключевые слова: генетическая инженерия, геномное редактирование, трансген, методы доставки, *floral dip*

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Введение

Совершенствование методов молекулярной биологии, обеспечивающих исследователей возможностью манипулировать молекулами ДНК, объединяя фрагменты экзогенных ДНК различного происхождения в генетические конструкции для экспрессии в различных системах, привело к созданию нового направления – генетической инженерии растений. Прошло немногим более сорока лет с момента создания первого трансгенного растения *(Nicotiana tabacum* L.), в геном которого был перенесен химерный ген, включающий последовательности генов, кодирующих ферменты октопинсинтазы и хлорамфеникол-ацетилтрансферазы [1]. На примере первого трансгенного растения исследователям стало очевидно, что транскрипционно-трансляционный аппарат растительной клетки способен поддерживать транскрипцию, трансляцию и посттрансляционные модификации перенесенных из других гетерологичных систем генов и продуктов их экспрессии. Именно это послужило отправной точкой для создания и развития агробиотехнологий, позволяющих модифицировать растительные геномы с целью улучшения хозяйственно-ценных характеристик у важных сельскохозяйственных видов растений. Известны примеры создания трансгенных растений, устойчивых к гербицидам, различным видам болезней, поражению насекомыми-вредителями, устойчивых к различным неблагоприятным абиотическим факторам среды, а также с улучшенными характеристиками пищевой ценности и качества производимой из них продукции [2, 3, 4]. Модификация метаболических путей биосинтеза некоторых вторичных соединений у растений послужила основой для создания «золотых бананов» и «золотого риса» с повышенным уровнем β-каротина, являющегося предшественником витамина А [5, 6], томатов с содержанием в плодах сквалена, фитостеролов, α-токоферола и каротиноидов [7], а также растений риса и сорго с более высоким содержанием цинка, железа и фолиевой кислоты [8]. Включение в состав кассеты экспрессии генов, кодирующих антигены возбудителей различных инфекционных заболеваний, а также тканеспецифичных регуляторных элементов, обеспечивающих их экспрессию в съедобных частях растения, привело к выдвижению концепции «съедобной вакцины» [9], а также привлекло внимание к таким генетически модифицированным растениям как источникам «съедобных рекомбинантных вакцин» [10, 11]. Модификация геномов растений с применением технологий генной инженерии открыла широкие перспективы в области создания биопродуцентов рекомбинантных белков, включая биофармацевтику [12]. Все больше внимание специалистов привлекает производство моноклональных антител, в том числе и противораковых, в растениях, поскольку растительные системы могут быть более дешевыми, безопасными и масштабируемыми, чем системы экспрессии клеток млекопитающих, дрожжей, бактерий и насекомых [13, 14]. Ожидаемое увеличение рыночного спроса на высокоэффективные и более доступные терапевтические моноклональные антитела растительного происхождения повышают коммерческий интерес к платформам экспрессии на растительной основе, обеспечивая тем самым увеличение объемов инвестиций и активное привлечение крупных фармацевтических компаний к совместной разработке новых препаратов [15].

Диапазон применения рекомбинантных нефармацевтических белков, синтезируемых в тканях трансгенных растений, также достаточно широк. Современное производство

рекомбинантного трипсина основано на его биосинтезе в семенах кукурузы [16]. Известны примеры использования рекомбинантных белков животного происхождения, синтезируемых растительными клетками, таких, как коллаген, кератин, шелк и эластин, которые обладают высокой прочностью, жесткостью, эластичностью и биосовместимостью и, следовательно, могут использоваться для производства новых и устойчивых биополимеров [17]. В Китае были коммерциализованы и получили сертификат биобезопасности трансгенные растения кукурузы, в зернах которых накапливался рекомбинантный фермент фитаза, способная расщеплять фитаты, что улучшает переваримость растительных кормов [18].

Следует подчеркнуть, что за четыре десятка лет с момента появления первого трансгенного растения, технология модификации геномов растений с применением методов генной инженерии продемонстрировала свою успешность в создании сортов с улучшенными характеристиками, а также биопродуцентов рекомбинантных белков. Однако сложность и высокая стоимость этой технологии, включающей широкомасштабные отборы благоприятных событий интеграции генетических конструкций в геномы растений, а также некоторое настороженное отношение со стороны человеческого сообщества стимулировали необходимость поиска более простых путей целенаправленной модификации генома, что и послужило следующим этапом в развитии методов модификации растительных геномов с применением геномного редактирования.

В настоящее время геномное редактирование широко используется с применением CRISPR/Cas9 как усовершенствованный метод трангенеза для получения мутаций по многим хозяйственно-ценным признакам у растений, в том числе и таким, как устойчивость к неблагоприятным факторам среды, ответным реакциям растений на стрессовые воздействия и т.д. [19]. Известны работы по улучшению профиля гликозилирования путем нокаутов генов *XylT* и *FucT*, *кодирующих ферменты* α(1,3)-фукозилтрансферазу и β(1,2)-ксилозилтрансферазу в клеточной культуре табака [20, 21]. Улучшение хозяйственно-ценных признаков у сельскохозяйственных видов растений с применением методов геномного редактирования представлено в обзоре [22]. Рассмотрим основные особенности модификации генома растений с применением методов генной инженерии и геномного редактирования по сравнению с традиционными методами селекции с акцентом на методы доставки экзогенных ДНК как наиболее важного этапа этих технологий.

Особенности модификации геномов растений

Комбинаторный принцип модификации генов и индукция случайных мутаций

В целом, ретроспективно рассматривая приемы адаптации и улучшения хозяйственно-ценных признаков у важных сельскохозяйственных видов растений, можно выделить две тенденции, одна из которых основана на интуитивном отборе отдельных форм, несущих спонтанные мутации, и вторая – на целенаправленном внесении мутаций в улучшаемые формы растений.

В первом случае основой будущего нового сорта послужит дальнейшее поддержание и сохранение среди потомков отобранных форм растений путем их переопыления.

Очевидно, что такой подход основан на комбинаторном принципе модификации генов и определяется искусством селекционера-исследователя не только обнаруживать необходимые для улучшения нового сорта признаки, но и закреплять их в новом создаваемом сорте. Селекционерами было разработано до¬статочно большое число методов и приемов, по¬зволяющих комбинировать гены, участвующие в проявлении хозяйственно-ценных признаков у растений, и отбирать формы с максимальным их проявлением. В настоящее время такие методы отбора дополнены современными методами молекулярного анализа с использованием молекулярных маркеров, таких, как SSR, SCAR, SNP и др., которые наследуются сцеплено с какими-либо важными хозяйственно-ценными характеристиками и позволяют селекционеру, ориентируясь на выявленные маркеры, насыщать генотипы будущего создаваемого сорта полезными признаками [23, 24]. Поиск и выявление QTL-генов, обеспечивающих сцепленное наследование сложных количественных признаков, таких, как, например, урожайность или засухоустойчивость [25, 26], также обеспечивает насыщение геномов растений будущего сорта генами, вносящими вклад в их проявление.

Вторая тенденция в направлении улучшения хозяйственно-ценных признаков у растений основана на попытках ускорения частоты возникновения мутаций. Возможность внесения мутаций в геномы различных видов растений появилась с открытием группы факторов физической (различные виды излучений) и химической природы (химические мутагены), позволяющих исследователям индуцировать двуцепочечные разрывы в молекулах ДНК. С точки зрения молекулярной биологии такие мутации представляют собой нарушения в последовательности ДНК, возникающие при восстановлении двуцепочечных разрывов с помощью ферментов репарации растительной клетки. Именно восстановление поломок, индуцированных ферментами репарации, и приводит к образованию широкого спектра различных перестроек (точечные мутации, делеции, ин¬версии, инсерции, транслокации и т. д.), которые могут быть ассоциированы с улучшением каких-либо признаков у растений, в том числе и хозяйственно-ценных.

Следующим этапом в развитии методов модификации геномов растений с помощью внесения различного рода мутаций, послужили методы, основанные на применении технологий рекомбинантных ДНК, позволяющих вносить в геном растения-донора фрагменты экзогенных ДНК, включающих кассеты экспрессии с целевыми генами. С точки зрения генетики такие экзогенные фрагменты ДНК, интегрированные в геном растения-донора (трансгенного растения), рассматриваются как инсерционные мутации. Более того, дальнейшее развитие этих методов привело к разработке молекулярных инструментов, основанных на открытии специфических участков у бактерий (CRISPR/Cas), позволяющих целенаправленно вносить двуцепочечные разрывы в выбранные исследователем целевые районы-мишени и, таким образом, индуцировать в них мутации.

Модификация геномов с применением технологий рекомбинантных ДНК

Случайный характер распределения экзогенных ДНК в геноме растения-донора при использовании методов генетической инженерии

В основе методов, основанных на технологиях рекомбинантных ДНК, лежит создание искусственных генетических конструкций, включающих различные гены из

разных гетерологичных систем. Такой подход снимает природные ограничительные барьеры между видами и царствами живых организмов и позволяет исследователям манипулировать с различными источниками экзогенных ДНК. Однако первые попытки переноса экзогенных ДНК в геном растительной клетки столкнулись с надежным барьером - целлюлозной оболочкой, защищающей клетки от проникновения чужеродных источников генетической информации. Использование исследователями методов биобаллистики с нанесением на частицы золота или вольфрама фрагментов экзогенных ДНК [27], а также протопластов, у которых целлюлозная оболочка разрушалась специальными целлюлитическими ферментами, позволило преодолевать эти барьеры и доставлять созданные генетические конструкции в цитоплазму и ядро растительной клетки [28]. Широкое распространение получили и методы, основанные на природной способности поч¬венной бактерии A. tumefaciens доставлять часть своих генов в геном растения. Перенос в геном растительных клеток части своей ДНК, обозначаемой как Т-ДНК, является этапом жизненной стратегии A. tumefaciens, нацеленной на биосинтез необходимых питательных веществ (опинов) за счет транскрипционно-трансляционной ма¬шины и ресурсов растительной клетки. Доставленные в ядро растительной клетки трансгены попадают в открытые петли хромати¬на и ферментами репарации включаются в восстанавливаемые места разрывов в ДНК по механизму негомологичной рекомбинации [29, 30]. Таким образом, в результате замены собственных генов в составе плазмиды A. tumefaciens на искусствен¬но созданные генетические конструкции был получен инструмент для их доставки в растител¬ный геном. Следует подчеркнуть, что доставленные в геном растения фрагменты экзогенных ДНК интегрируются в геноме случайным образом [31] и дальнейшая «судьба» перенесенных трансгенов будет во многом определяться генетическим окружением того района генома, в который произошла интеграция [32]. Более того, фрагменты экзогенных ДНК могут быть случайным образом интегрированы в районы расположения собственных генов и вызывать их изменения – Т-ДНК-индуцированные мутации [33, 34]. На основании вышеперечисленных особенностей становится очевидным, что технология модификации геномов растений с применением методов генетической инженерии достаточна сложна и подразумевает поиск благоприятных событий интеграции экзогенных ДНК, экспрессия целевых генов в которых будет сохраняться на высоком уровне в течение последующих поколений и не будет связана с мутационными изменениями других генов, снижающих характеристики хозяйственно-ценных признаков.

Возможность выбора районов-мишеней в геноме растения-донора при использовании методов геномного редактирования

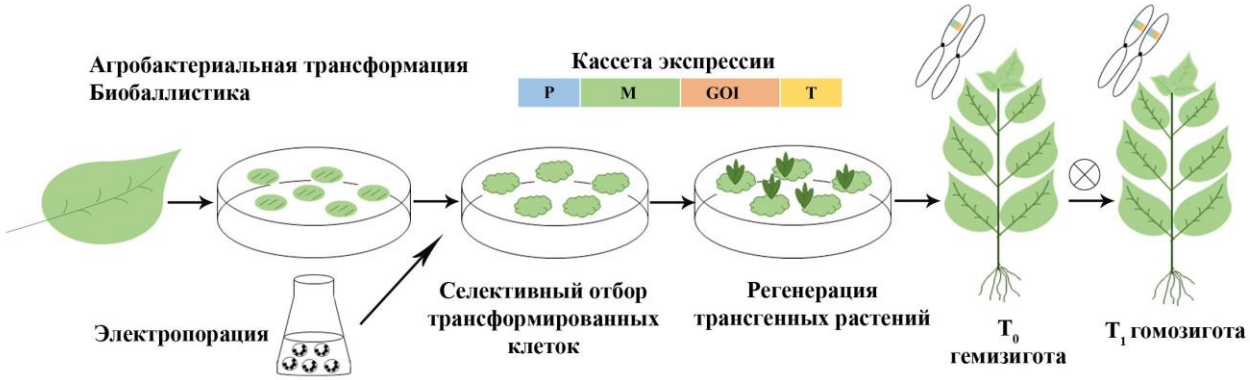
Основой для создания инструментов, позволяющих исследователям целенаправленно вносить двуцепочечные разрывы в выбранном районе-мишени растительного генома, послужило открытие у бактерий механизма борьбы с вторжением чужеродной ДНК бактериафагов [35]. Суть создания инструмента редактирования генома состоит в том, чтобы в состав генетической конструкции, переносимой в геном растительной клетки, был включен участок ДНК, комплементарный району гена-мишени, в котором планируется произвести двуцепочечный разрез. В качестве “молекулярных ножниц” для осуществления таких разрезов выступают нуклеазы, в частности, нуклеаза Cas9

из *Streptococcus pyogenes.* Следует подчеркнуть, что такие инструменты могут быть интегрированы в геном растения в виде наработанных в другой системе экспрессии рибонуклеиновых комплексов [36]. Генетическая конструкция, несущая в своем составе последовательность гена нуклеазы Cas9 и последовательность, гомологичную району гена-мишени, может быть интегрирована в геном с помощью разработанных и широко используемых способов агробактериальной и биобаллистической доставки.

Сравнительный анализ методов доставки экзогенных ДНК в ядерный геном растений

Методы, включающие этап культивирования in vitro

Технологии модификации генома растений с применением методов генетической инженерии и геномного редактирования на ранних этапах их формирования и дальнейшего совершенствования включали процесс дезинтеграции растительных тканей с последующим их культивированием *in vitro*. Необходимость включения этой стадии диктовалась особенностями организации клеточной стенки, представляющей собой сложный барьер для проникновения почвенных бактерий, например, *A. tumefaciens,* способных проникать в растительные клетки только в местах их повреждений. На рисунке 1 представлена общая схема доставки экзогенных ДНК в составе кассеты экспрессии в геном растительной клетки и получение гомозиготных по встроенным генам растений-трансформантов (Т1).



Примечание: P – промотор; M – последовательность маркерного гена; GOI – последовательность целевого гена *(gene of interest);* T – терминатор.

Рисунок 1. Схема доставки экзогенных ДНК (кассеты экспрессии) в ядерный геном растений, включающая этап культивирования клеток *in vitro*

Первые эксперименты по доставке экзогенных ДНК в ядерный геном растений были направлены на инокуляцию листовых эксплантов суспензией *A. tumefaciens.* После инокуляции листовые экспланты помещали на питательные среды с добавлением фитогормонов и соответствующих антибиотиков для селективного отбора трансформированных клеток и индуцировали из них каллусы с последующим

восстановлением из них полноценных растений-трансформантов. С помощью методов биобаллистики также можно было преодолеть барьерные препятствия клеточной стенки, помещая фрагменты экзогенных ДНК на поверхности золотых или вольфрамовых частиц и с помощью генной пушки доставлять их в ядро клетки. Наконец, исследователи попытались растворять клеточную стенку с помощью ферментов, однако, несмотря на успешную доставку кассет экспрессии при таком способе, дальнейшие процедуры восстановления растений из протопластов представляли собой чрезвычайно сложную задачу.

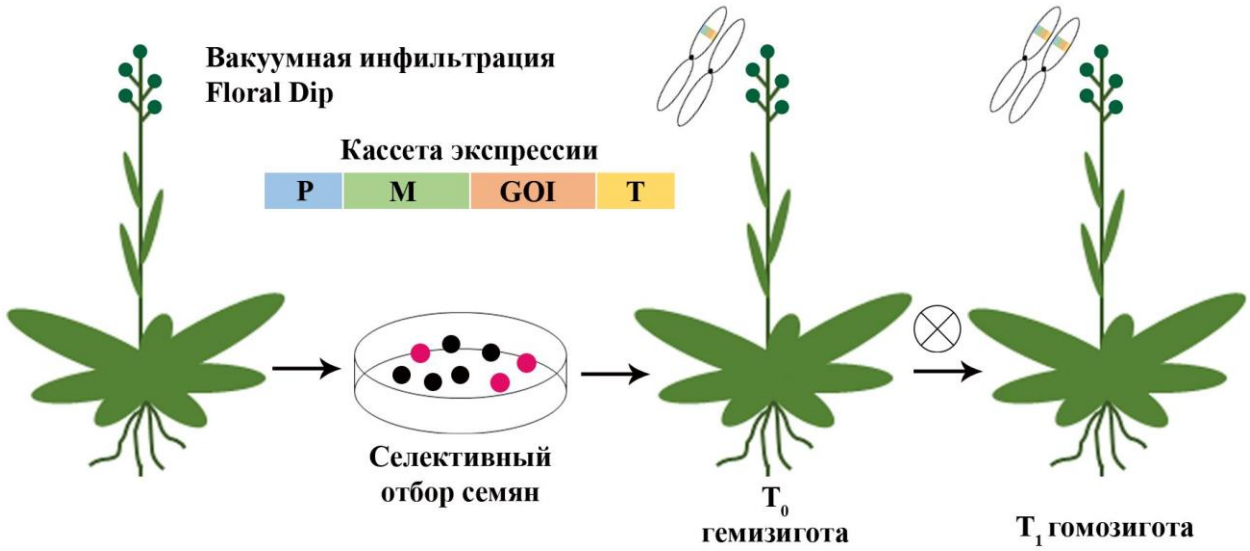
Итак, несмотря на успешность разработанной технологии получения генетически модифицированных растений, со временем стало очевидно, что эта технология достаточно сложна, трудоемка, требует много времени на рутинные работы от начала переноса трансгенов до получения трансформированных растений. Более того, этап культивирования клеток *in vitro* сопровождался сомаклональной изменчивостью, что не всегда рассматривалось как желаемое событие. Следует подчеркнуть, что несмотря на введение в культуру *in vitro* более двух сотен видов растений, для некоторых из них процедура регенерации все еще остается трудно разрешимой задачей. В связи с этим исследователи предприняли широкий поиск возможных вариантов доставки экзогенных ДНК непосредственно в геном растительных клеток, минуя их переход в состояние *in vitro.* К настоящему моменту такие методы были успешно разработаны, и продолжается их адаптация для различных видов растений.

Методы доставки экзогенной ДНК *in planta*

Метод доставки экзогенной ДНК в геном растительной клетки, минуя стадию культивирования in vitro, был разработан к 1987 году для *A. thaliana* [37]. Суть этого метода заключалась в том, что предварительно замоченные и набухшие семена *A. thaliana* помещали в суспензию *A. tumefaciens* на сутки, после чего отмывали водой в стерильных условиях, помещали на селективные среды с антибиотиком и отбирали трансформированные растения. На основании полученных данных стало очевидным, что активно делящиеся меристематические клетки зародыша становятся компетентными для агробактериального инфицирования. В дальнейшем этот процесс был существенно усилен за счет проведения агробактериального заражения эксплантов в условиях вакуумной инфильтрации [38]. Примечательно, что прорыв в направлении усовершенствования методов доставки экзогенных ДНК в геном растительных клеток, связанный с существенным упрощением этой процедуры и исключением этапа культивирования растительных клеток in vitro, оказал огромное влияние на молекулярно-генетические исследования с применением *A. thaliana* как модельного растения.

Следующим шагом в развитии методов вакуумной инфильтрации клеток эксплантов трансформируемых растений без культивирования и регенерации *in vitro* послужило простое погружение развивающихся цветочных побегов в агробактериальную суспензию с добавлением сахарозы и поверхностно-активного вещества Silwet L-77 [39]. Более того, авторами этого исследования было показано, что повторная обработка цветочных побегов, а также их укрытие в пакеты из пленки, увеличивало скорость трансформации и общий выход растений-трансформантов. Положительный эффект на увеличение

выхода трансформантов оказало воздействие на растения после обработки цветочных побегов стрессовыми факторами (засуха) [40]. К настоящему времени в литературе можно найти довольно много различных вариантов метода *floral dip* для *A. thaliana* [41, 42]. На рисунке 2 в самом общем виде представлены основные этапы этого метода.



Примечание: P – промотор; M – последовательность маркерного гена; GOI – последовательность целевого гена *(gene of interest);* T – терминатор; Красный цвет – семена с геном красного флюоресцирующего белка.

Рисунок 2. Схема доставки экзогенных ДНК (кассеты экспрессии) в ядерный геном растений методами *in planta*

Как хорошо видно на представленном рисунке, фрагменты экзогенных ДНК, объединенные в кассете экспрессии, могут быть доставлены непосредственно в геном целого растения с применением метода *floral dip* или в отдельные его части с использованием подхода вакуумной инфильтрации. Включение в состав кассеты экспрессии репортерного гена RFP, кодирующего красный флюоресцирующий белок под управлением промотора, активирующегося в семенных оболочках, позволяет проводить предварительный отбор уже на стадии семян, собранных с цветочных побегов после агробактериальной обработки и получать To (гемизиготы). После самоопыления гемизигот To среди потомков можно отобрать гомозиготные по целевому гену растения.

Несмотря на то, что доставка экзогенных ДНК методом *floral dip* достаточно широко применяется для *A. thaliana,* а также разработаны и продолжают разрабатываться модификации этого метода для других видов растений, полная картина механизма агробактериального заражения неповрежденных и, более того, достаточно удаленных от места нанесения агробактерий целевых тканей, все еще остается не вполне ясной. Исходя из получаемых результатов, свидетельствующих о том, что экзогенные ДНК обнаруживаются в тканях семенного зародыша, возможно предположить два пути их проникновения в целевые клетки. С одной стороны, экзогенные ДНК могут быть

интегрированы в пыльцевое зерно и затем в спермий, что представляется вполне логичным, поскольку пыльцевые зерна на поверхности пестика непосредственно контактируют с клетками агробактерий. С другой стороны, можно предположить и путь инфицирования яйцеклеток или зигот при их слиянии со спермиями, однако в этом случае остается неясным достаточно удаленный путь инфицирования яйцеклеток или зигот, поскольку они расположены внутри семяпочки. Первой работой, отчасти пролившей свет на механизм агробактериального заражения тканей цветка при использовании метода *floral dip*, является исследование тканей цветка *A. thaliana* при доставке гена *uidA,* кодирующего фермент бета-глюкуронидазу [43]. Авторами этого исследования было установлено, что активность фермента наблюдалась только в развивающихся семяпочках и не обнаруживалась в пыльце и пыльцевых трубках. Более того, было показано, что скорость трансформации семяпочек была в 6 раз выше у растений с открытой формой гинецея, что послужило основанием для утверждения, что агробактерии проникают к семяпочкам через внутреннюю часть открытого развивающегося гинецея.

Дальнейшее развитие метода *floral dip* для доставки экзогенных ДНК в ядерный геном других видов растений

Эффективность и простота применения метода *floral dip* для доставки фрагментов экзогенных ДНК (кассет экспрессии или инструментов для геномного редактирования) были весьма привлекательны для применения к другим видам растений. Именно это обстоятельство стимулировало широкий поиск возможностей его адаптации к различным видам растений (Таблица 1).

Таблица 1

Применение метода *floral dip* для доставки экзогенных ДНК в геномы различных видов растений

Вид растения	Семейство	Ссылка на источник
Medicago truncatula (люцерна усеченная)	*Fabaceae*	44
Brassica napus (рапс), *B. carinata* (горчица абиссинская)	*Brassicaceae*	45
Triticum aestivum (пшеница мягкая)	*Poaceae*	46
Zea mays (кукуруза обыкновенная)	*Poaceae*	47
Linum usitatissimum (лен обыкновенный)	*Linaceae*	48
Tagetes erecta (бархатцы мелкоцветковые)	*Asteraceae*	49
Solanum licopersicum (томаты)	*Solanaceae*	50
Cosmos sulphureus (космея серно-желтая)	*Asteraceae*	51
Descurainia Sophia (Дескурайния Софии)	*Brassicaceae*	52

Как видно из таблицы 1, к настоящему времени в литературе имеются сведения об успешном применении метода *floral dip* для 10-ти видов растений, являющихся представителями 6-ти семейств [44-52]. Принимая во внимание особенности строения

цветков, представителей перечисленных в таблице видов растений, следует отметить, что они существенно различаются между собой, однако, как показывают результаты экспериментов, доставка экзогенных ДНК с применением метода *floral dip* может быть для них успешной. Данное обстоятельство вселяет оптимизм на расширение приведенного списка в случае необходимости получения генетически модифицированных видов растений с применением методов генной инженерии и геномного редактирования, и в особенности для тех видов, для которых все еще не разработаны протоколы культивирования *in vitro.* Необходимо отметить, что на поиск методов и подходов для доставки экзогенных ДНК в геном растительных клеток in planta в настоящий момент времени направлены усилия многих исследовательских коллективов [53].

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Настоящая статья не содержит описания выполненных авторами исследований с участием людей или использованием животных в качестве объектов.

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Генетикалық инженерия және геномдық өңдеу әдістері мен өсімдік геномдарының модификациясы: экзогендік ДНҚ жеткізу

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Аңдатпа. Биологияның постгеномдық дәуірінде әртүрлі өсімдік түрлерінің геномдарына экзогендік ДНҚ фрагменттерін жеткізу әдістерін дамыту мен жетілдірілу туралы деректер қарастырылады. *In vitro* жағдайында өсімдік жасушаларын өсіру сатысына байланысты экзогендік ДНҚ-ны жеткізу ерекшеліктері келтірілген. Өсімдік ұлпаларының жасуша культураларына ыдырауын болдырмайтын, содан кейін трансформаторлық – өсімдіктерді қалпына келтіретін, яғни *in vitro* кезеңін айналып өтетін, *in planta* өсімдік жасушаларының геномдарына экспрессиялық кассеталарды жеткізуді қамтамасыз ететін әдістерді дамытуға баса назар аударылады. *Arabidopsis thaliana* модельді өсімдіктері үшін де, жоғары сатыдағы өсімдіктердің алты тұқымдасының өкілдері болып табылатын кейбір басқа түрлер үшін де *floral dip* әдісін қолданудың табыстылығы туралы деректер берілген. Геномдық өңдеу әдістерімен өсімдіктерді модификациялауға қызығушылықтың артуына байланысты *floral dip* әдісінің сұранысы туралы деректер келтірілген.

Түйін сөздер: генетикалық инженерия, геномдық редакциялау, трансген, жеткізу әдістері, *floral dip*

Modification of Plant Genomes by Genetic Engineering and Genome Editing: Delivery of Exogenous DNA

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Abstract. The article reviews data on the development and improvement of methods for delivering exogenous DNA fragments into the genomes of various plant species in the postgenomic era of biology. The features of exogenous DNA delivery, depending on the stage of plant cell cultivation *in vitro* are presented. The emphasis is placed on the development of methods that ensure the delivery of expression cassettes *into plant* cell genomes in planta, excluding the disintegration of plant tissues to cell cultures with subsequent restoration of transformant plants, i.e., bypassing the in vitro stage. Data are presented on the success of the *floral dip* method for both model plants *Arabidopsis thaliana*, and some other species that are representatives of six families of higher plants. Data are presented on the demand for the *floral dip* method in connection with the growing interest in plant modification by genome editing methods.

Keywords: genetic engineering, genome editing, transgene, delivery methods, *floral dip*

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Role of anthocyanins in plant resistance to virus

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Abstract. Viral infections pose a serious threat to crop production in Kazakhstan and worldwide, negatively affecting the growth, development, and productivity of agricultural crops. Under conditions of multiple stresses, such as drought, extreme temperatures, soil salinity, and pathogen damage, viruses aggravate physiological changes in plants, disrupting their metabolic pathways and reducing resistance to adverse factors. Particular attention is paid to the effect of viral infections on the biosynthesis of anthocyanins, important compounds involved in plant defense mechanisms. In this work, it was shown that infection with tomato bushy stunt virus (TBSV) caused more damage to the middle leaves of the model plant compared to other leaves, and the upper leaves stopped developing. In addition, necrosis was observed in the middle leaves, which led to further programmed cell destruction (PCD). Moreover, infection with the TBSV virus led to a significant increase in hydrogen peroxide levels and accumulation of anthocyanins in *Nicotiana benthamiana* plants. These changes indicate a disturbance in the redox balance and activation of defense reactions in response to viral infection. The findings highlight the importance of studying the interaction of viruses with plants to develop strategies to improve crop resistance to viral infections and other stress factors.

Keywords: *N. benthamiana,* virus, TBSV, biotic stress, ROS, H_2O_2, anthocyanin

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Introduction

Viral infections pose a significant threat to agricultural production and can cause significant damage to global food security [1]. The development of viral infections in economically important crops over several generations leads not only to yield losses caused directly by the viruses themselves but also to degradation or degeneration of varieties, thereby increasing the harmful effects. In general, this phenomenon can lead to yellowing of leaves, changes in their shape and height of stems, and a decrease in vegetative mass and quality of harvest [2]. One of the deadly affecting causes is viral invasion of plant tissue, which leads to necrosis and programmed cell death (PCD). Plants experience a variety of metabolic and physiological changes during stress, including changes in shoot/root biomass, reduced photosynthesis and nutrient intake, and suppression of flowering and seed development, all of which contribute to decreased growth and productivity [3].

Tomato bushy stunt virus (TBSV) is a member of the Tombusviridae family and possesses a single-stranded positive RNA genome of about 4800 nucleotides encased by 180 capsid protein subunits [4]. TBSV is composed of 30 nm-diameter spherical particles with a positive-sense single-stranded RNA genome of about 4.8 nt that encodes five main open reading frames (ORFs). ORF1 and ORF2 are necessary for viral replication. ORF3 encodes the coat protein, whereas ORF4 encodes the viral movement protein, which is required for cell-to-cell mobility and symptom detection on particular host plants. ORF5 products play a role in inducing necrotic signs as well as the virus's long-distance transmission, depending on the host [5].

The virus has five unique open reading frames: p33 and p92 for the replicase, which are translated from genomic RNA; P41, the capsid protein, is translated from subgenomic RNA 1, while P22 and P19 are translated from subgenomic RNA 2. P19 is the key determinant of the virus's pathogenicity, and it suppresses RNA interference-based defensive systems [6,7].

This virus spreads naturally through contaminated seeds and propagative material, as well as through manual use of infected cutting equipment [8]. TBSV has a limited host range, affecting just a few dicotyledonous species from several families. It also affects numerous vegetable crops [9].

TBSV causes stunting, bushy growth, distortion, and necrosis in tomatoes, eggplants, and peppers. Fruits of infected plants develop necrosis and chlorotic blotching, causing significant economic damage such as production loss and decrease in the quality of commercial solanaceous crops farmed in greenhouses and fields [5].

Therefore, there is an urgent need to study in detail the defense mechanisms used by plants against viral infections. Reactive oxygen species (ROS) play a crucial role in responding to viral infections by integrating various signalling networks and activating plant defense mechanisms [10].

Reactive oxygen species (ROS) are obligatory products of metabolism in living organisms and have several types of these molecules, including the superoxide radical (O_2•–), hydrogen peroxide (H_2O_2), hydroxyl radical (•OH), singlet oxygen ($^1O_2$), peroxy radical (ROO•), and alkoxyl radicals (RO•) [11–15]. ROS production happens in plant tissue as a consequence of cell wall peroxidases, amine oxidases, NADPH oxidases, oxalate oxidases, lipoxygenases, and quinone reductase [16,17]. ROS accumulated in plant tissue under stress conditions, such as high temperature, salinity, low temperature, and biotic stresses such as virus invasion, bacterial infection, and other agents [18,19]. Excessive ROS accumulation negatively affected plant tissue

and led to significant damage, which was described as oxidative damage [19]. Nucleic acid degradation, carbohydrate, protein, and lipid peroxidation that lead to PCD.

ROS accumulation serves as a signalling molecule to activate the defense system of plants, such as non-enzymatic (tocopherol, proline) and enzymatic (ascorbate peroxidase, catalase, superoxide dismutase, peroxidase, GSH) ROS scavenging molecules [20–22]. Furthermore, reactive oxygen species damaged the photosynthetic apparatus of plants by reducing photosynthesis efficiency, stomatal conductance, and chlorophyll content [23].

Moreover, during the virus infection, Sugarcane mosaic virus (SCMV) in transgenic sugarcane was detected at a low level of ROS concentration; meanwhile, another hybrid of sugarcane showed a higher level of ROS and MDA, which are evidence of disruption of cell ultrastructure [18]. Between virus infection and ROS level, there are strong bounds, and in tobacco infected, it has determined the uricase activity, which is mainly affected by hydrogen peroxide production [24–27]. Also, cowpea cultivars treated by the ringspot virus showed higher accumulation of superoxide and superoxide dismutase activity, which serves as a converter of superoxide to hydrogen peroxide [24]. Additionally, higher levels of ROS and lipid peroxidation and protein oxidation leading to Sharka symptoms were a consequence of long-term infection of the *Plum pox virus* in *Prunus* species [25].

The increasing amount of H_2O_2 triggers the production of anthocyanin under various stresses. Anthocyanins are antioxidant compounds that help to mitigate oxidative stress by neutralizing reactive oxygen species (ROS) like H_2O_2. Besides, H_2O_2 might activate transcription factors controlling the expression of genes responsible for anthocyanin biosynthesis, including MYB, bHLH, and WD40 transcription factors; therefore, activation of these factors is significant for anthocyanin pathways [26]. Also, H_2O_2 interacts with plant hormones, including ABA, JA, and ethylene, which are known modulators of anthocyanin accumulation [27]. Many of these hormones also are induced by stress, again leading to coordinately responsive outcomes in which anthocyanin synthesis is one of the consequences.

To address this issue, plants have developed a variety of ROS scavenging mechanisms, including the induction of anthocyanin molecules, that provide protective benefits under stress [28]. One approach for conferring stress resistance on plants is the accumulation of anthocyanins. Anthocyanins are water-soluble flavonoid chemicals that give flowers, fruits, and vegetables characteristic red, purple, orange, blue, and brown colors [29]. Under stress, anthocyanin molecules accumulate in many plant tissues, providing considerable antioxidant activity and inducing plant morphophysiological and metabolic responses [30]. Pigmented leaves and crops aid in stress tolerance by increasing ROS scavenging [31]. Furthermore, different levels and concentrations of anthocyanins contribute to plant biodiversity and adaptation, with pigmented plants containing higher anthocyanin levels being more tolerant to various stress conditions and beneficial for human nutrition [32].

For example, significant anthocyanin accumulation was observed under salt stress in wheat genotypes of different colors [29]. In another case, grapevine cells under phosphate deficiency showed anthocyanin accumulation responsible for stress tolerance [33]. Similar observations of anthocyanin biosynthesis gene induction under stress conditions have been reported in various crops, conferring stress tolerance [34]. Given their antioxidant properties, anthocyanins

protect plants from adverse environmental conditions by scavenging stress-induced ROS and are considered a promising approach to enhancing plant stress tolerance [35]. However, the role of anthocyanins in the stress pathogen response remains underexplored.

This study aims to investigate the impact of viral infections on anthocyanin accumulation in plants. Understanding how viruses interact with the metabolic pathways responsible for anthocyanin synthesis can provide valuable insights into plant defense mechanisms and aid in the development of more resilient crop varieties.

Materials and research methods

Grows of N. benthamiana

The *Nicotiana benthamiana* plants used in this study were cultivated in enriched soil (Volshebnaya Gryadka, produced in Russia) within a controlled growth room under artificial lighting conditions. To simulate optimal growth and development conditions, a long-day photoperiod was established using alternately installed lamps with spectra of 2700 K and 6400 K, providing 16 hours of light (day) and 8 hours of darkness (night). Seeds were placed in soil pre-moistened with distilled water, and seedlings were transferred into new individual pots 10-14 days following germination, as they grew. To avoid contamination, the planting pots were sterilized with disinfectant treatments. The growth room had a relative humidity of 75-80% and an air temperature of 23-27 ºC. Watering was conducted three times a week with a consistent amount of distilled water at the same time each day.

Preparation of material for inoculation

Plasmids containing TBSV cDNAs were linearized with SmaI type II restriction endonuclease from ThermoFisher Scientific according to the manufacturer's instructions. The restriction products were then purified by phenol-chloroform extraction with subsequent ethanol precipitation. The linearized plasmids served as a template for the synthesis of TBSV viral RNA transcripts. The reaction was carried out using a set of ribonucleotide mixture, reaction buffer, and T7 polymerase from ThermoFisher Scientific. The in vitro transcription products were separated and visualized in 1% agarose gel.

Horizontal agarose gel electrophoresis

Separated in 1% agarose gel with ethidium bromide for 40 minutes with buffer. An agarose gel was prepared using a ratio of 500 mg agarose to 50 ml of buffer containing TRIS, boric acid, and EDTA (TBE). Viral particles were detected on agarose gels using UV light. The presence of DNA in the gel was detected under ultraviolet light using a Vilber Lormat gel documentation system (France).

Inoculation with viral material

Inoculation was carried out by the rub-inoculation method. The transcripts obtained in vitro were mixed with phosphate buffer and carborandrum. For inoculation, 2-3 leaves from the middle tier were selected. Mechanical damage was caused by light movements, through which

matrix RNA penetrates into plant tissues and cells. N. benthamiana plants aged 30-35 days were inoculated with in vitro synthesized TBSV transcripts.

Immunodetection of viral proteins

To determine the presence of viral proteins, hydroxyapatite chromatography fractions from healthy and virus-infected plants were separated on 15% polyacrylamide gels and transferred to nitrocellulose membrane (Osmonics, Westborough, MA). Transfer efficiency was tested by incubating the membrane in Ponceau S solution (Sigma, St. Louis, MO). Membranes were incubated with anti-capsid protein antibody solution (1:5000) for 2 h. After washing three times, secondary antibodies conjugated to alkaline phosphatase 1:3000 were added. NBT-BCIP solution was used to visualize the formed immune complexes [36].

Detection of H_2O_2 in N. benthamiana

The upper non-inoculated leaves were examined to identify ROS components, such as H2O2, which are known for their detrimental effects on plant growth and development. For H2O2 detection, samples were extracted in 50 mM phosphate buffer (pH 7.5) at a ratio of 1:8 (w/v) and centrifuged twice at 10,000 rpm for 10 minutes. The reaction mixture for detecting H2O2 consisted of 0.85 mM 4-aminoantipyrine, 3.4 mM 3,5-dichloro-2-hydroxybenzene sulfonate, and 4.5 U/ml HRP in 2 ml of 50 mM phosphate buffer (pH 7.5) as previously described by Yesbergenova et al. [37]. Absorbance was measured after 5 minutes at 515 nm using a Spectrophotometer.

Quantitative determination of anthocyanin content

The upper, uninoculated leaves were sampled. Samples were homogenized with an Extraction buffer containing 45% methanol and 5% acetic acid in a ratio of 1:5. Then they were centrifuged twice at 4°C, 10,000 rpm for 10 minutes each time, and then placed on a special plate. Measured as described by Nakata and Ohme-Takagi [38]. Absorbance was measured at 530 and 637 nm in a microplate spectrophotometer, “Multiskan SkyHigh” (Thermo Fisher Scientific, USA).

Statistical analysis

Each treatment option was analyzed in triplicate samples from each plant. Statistical analysis was performed using the StatPlus Professional 5.8.4.3 2018 version for Windows software package (AnalystSoft Inc., www.analystsoft.com/ru/), Student's t-test. Values were expressed as mean ±SE. P values below 0.05 were considered statistically significant. If the null hypothesis is true, the group's assessment of dyspepsia with alcohol changes should be close to the assessment of maternal dyspepsia. It should not be similar.

Results

One-month-old *N. benthamiana* plants with initially similar morphological traits, such as height, leaf plate development, and total vegetative mass, were selected for inoculation. The plants were inoculated by treating the leaves of the middle tier with an inoculation mixture. *N. benthamiana* plants were infected in vitro with wild-type transcripts of TBSV.

Seven days after inoculation with *Tomato Bushy Stunt Virus* (TBSV), the infected plants developed characteristic symptoms indicating systemic spread of the virus and accumulation of viral proteins (Figure 1). Local chlorotic and necrotic spots and, in some cases, necrotic rings were observed on the inoculated leaves during the first 2–5 days. Some plants showed cell death at the inoculation site, which may indicate a hypersensitive response. Systemic symptoms of infection appeared on the 7th day after inoculation. Most plants showed pronounced chlorosis and mosaic coloration of the leaves, accompanied by their deformation (curling, size reduction, wrinkling). Inhibition of apical growth, shortening of internodes, and development of dwarfism were also recorded. In some cases, tissue necrosis was observed along the main veins of the leaves. The observed symptoms indicate active accumulation of viral proteins and successful systemic infection, which confirms the ability of TBSV to spread rapidly in the host plant.

Figure 1. Morphological signs of infection development in *N. benthamiana* plants

To confirm the presence of viral particles in N. benthamiana plants infected with wild-type TBSV, agarose gel electrophoresis and immunoblotting analyses were performed. Samples extracted from inoculated and healthy plants were subjected to 1% agarose gel electrophoresis followed by ethidium bromide staining. Visualization under UV light revealed a clear band corresponding to TBSV virions in samples obtained from infected leaves (Figure 2). To confirm the specificity of the detected particles, capillary transfer onto a nitrocellulose membrane was performed, followed by immunostaining with polyclonal antibodies specific for TBSV virion. The proposed methodological approach demonstrates high efficiency for rapid and reliable detection of TBSV virions in inoculated plants. This method can be particularly useful in cases where visual morphological signs of viral infection are weak or absent, making it a valuable tool for diagnosing viral infections in plants.

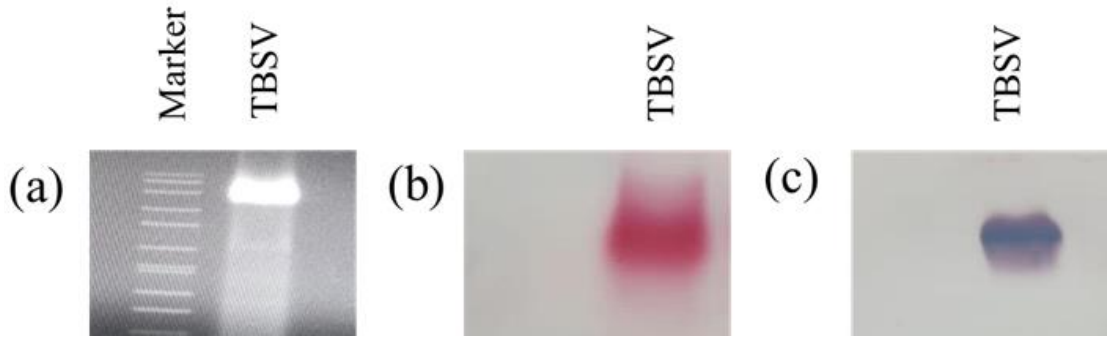
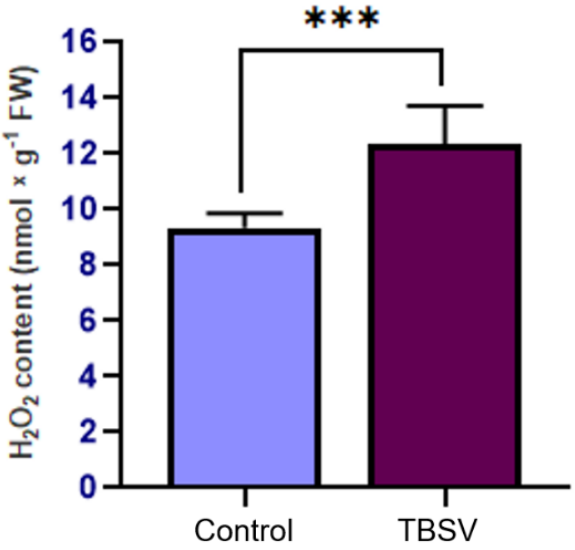


Figure 2. Detection of virion particles in *N. benthamiana* infected with wild-type TBSV. **(a)** Ethidium bromide staining of agarose gel after electrophoresis; **(b)** Ponceau S staining of nitrocellulose membrane; **(c)** Western blot analysis for detection of TBSV virions.

Reactive oxygen species (ROS) play a key role in plant signaling networks, participating in the regulation of many biological processes, including defense responses to pathogens. One of the most important representatives of ROS is hydrogen peroxide (H_2O_2), which, when accumulated in cells, can cause oxidative stress that affects the physiological state of the plant. In this study, we examined the effect of TBSV infection on the level of H_2O_2 accumulation in N. benthamiana plants.

To assess changes in ROS levels, experiments were conducted to quantify H_2O_2 in the tissues of infected and healthy plants. The results showed that TBSV infection leads to a significant increase in H_2O_2 concentration compared to control (healthy) plants (Figure 3).



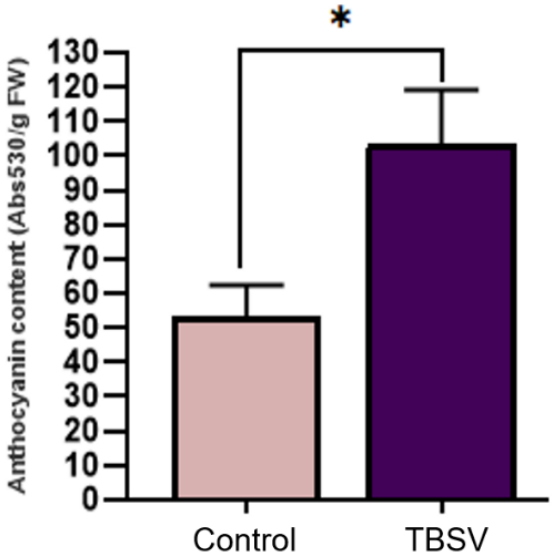
Note: Asterisks in the graph "***" indicate a very significant (P < 0.01); "ns" – an insignificant (P > 0.05) difference in the presented data. Statistical analysis (Student's t-test) was performed using GraphPad Prism software (v.8.01). Data are presented in relative units.

Figure 3. Determination of hydrogen peroxide accumulation in the leaves of *N. benthamiana*

The obtained data allow us to conclude that the increase in the accumulation of hydrogen peroxide (H_2O_2) in response to a viral attack may be one of the key components of the defense response of *N. benthamiana* plants. Hydrogen peroxide, as one of the most stable reactive oxygen species (ROS), plays an important role in the formation of an oxidative burst, which in turn contributes to the activation of additional plant defense mechanisms.

The interaction of the virus with the host plant is a complex process involving many physiological mechanisms, including the regulation of ROS levels, the functioning of the antioxidant system, and the accumulation of stress markers such as malondialdehyde (MDA), proline, and anthocyanins. The accumulation of ROS in response to a viral infection activates signaling cascades, which leads to the initiation of defense reactions. Anthocyanins probably act as ROS scavengers, protecting cells from oxidative damage and increasing plant resistance to pathogens. Therefore, the determination of the level of anthocyanin accumulation under the influence of TBSV was investigated.

As a result of the experiments, it was found that in *N. benthamiana* plants, in response to viral infection with wild-type TBSV, there is a significant accumulation of anthocyanin in the leaves compared to extracts obtained from control, uninfected plants (Figure 4). These results suggest that anthocyanins may play an important role in plant defense systems by being activated in response to virus infection and participating in the neutralization of oxidative stress. Thus, the accumulation of H_2O_2 and anthocyanins in response to virus infection is an important element of the defense response of *N. benthamiana* plants, highlighting their role in enhancing resistance to pathogens.



Note: Asterisks in the graph "***" indicate a very significant (P < 0.01); "ns" – an insignificant (P > 0.05) difference in the presented data. Statistical analysis (Student's t-test) was performed using GraphPad Prism software (v.8.01). Data are presented in relative units.

Figure 4. Determination of anthocyanin accumulation in the leaves of *N. Benthamiana*

Discussion

As a result of successful inoculation, plants exhibited TBSV-specific disease symptoms at 7 days post-inoculation, indicating systemic spread of the virus and accumulation of viral proteins (Figure 1). The middle leaves of model plant more damaged in comparison with other leaves, and the tops of the leaves stopped development. Additionally, in middle leaves has observed necrosis which leading to further PCD of plants.

In the majority of cases observed, the interaction between viruses and cultivated crop plants has a detrimental impact on host morphology and physiology, leading to diseases [39]. Similarly, in plants including *Lycopersicon esculentum L*. and *C. annuum*, infected by TYLCV observed the internode reduction, curling and dwarfing of leaves [40].

Plants have evolved complex signaling and defensive mechanisms to cope with stressful environments. One of the first plant reactions to pathogen invasion is a considerable increase in reactive oxygen species (ROS) levels [41]. TBSV infected plants showed the higher accumulation of hydrogen peroxide level in leaves in comparison with not infected plants. (Figure 2). Similar results where hydrogen peroxide over produced during infection of plum pox virus (PPV) in *Prunus armeniaca L.* and *Prunus persica L.* [42]. Furthermore, the hydrogen peroxide level strongly correlated with lipid peroxidation, electrolyte leakage and protein oxidation [43,44]. Excessive amount of hydrogen peroxide influenced to the activation of ROS scavenging enzymes including SOD. Interestingly, apoplastic SOD participated to generation of secondary cell wall [45]. Simultaneously, the as an ROS scavenging enzymes, the level of phenolic compounds including anthocyanin, increased response to oxidative damage and it pointing out the critical role of anthocyanin as antioxidative function [46].

As demonstrated our results, anthocyanin content increased during the infection with TBSV, whereas in control plants remains, whereas in control plants remains unchanged. Similarly, trends were observed in Grape leaves *(Vitis vinifera L.)* infected by *Grapevine leafroll-associated virus 3 (GLRaV-3).* It suggests that anthocyanin were sensitive to stress conditions than other phenolic compounds. Additionally, anthocyanin is one of the key players of the oxidative defense system in vivo [47]. Moreover, hydrogen peroxide accumulated in different parts of plants, whereas anthocyanin accumulated only in vacuoles, and it was determined in *Malus domestica Borkh*. In vitro ROS scavenging function of anthocyanin was affected actively than in vivo [47]. Also, during the stress condition, anthocyanin content accumulated in adaxial and abaxial cells, which are located near to meshophilic cells [48]. Grape leaves *(Vitis vinifera L.)* infected with grapevine leafroll disease (GLD) exhibited downward curling of leaf margins and increased anthocyanin biosynthesis, causing a reddish-purple coloration [49].

In recent years, there has been an increasing interest in researching the role of anthocyanins in plants, particularly in terms of their response to biotic stressors such as virus infections [46, 48]. Previous research has demonstrated that virus infection can boost anthocyanin levels in a range of plants [48]. These findings indicate that anthocyanins may play a significant role in plant defense systems by activating in response to viral infection.

Previously, Tsuyoshi Inukai investigated the effect of anthocyanins in *Brassicaceae* species infected with turnip mosaic virus [50]. It has previously been discovered that turnip mosaic

virus significantly reduces anthocyanin production in Brassica rapa leaves, and that such leaves become infected while anthocyanin-rich leaves on the same plants are rarely affected. The authors indicate that anthocyanin accumulation dramatically inhibited turnip mosaic virus infection, implying that it acts as a chemical barrier against the virus, demonstrating the protective role of anthocyanins [50].

Another study that demonstrates the link between viral infections and anthocyanin accumulation is that of Xiang-Ru Chen, who evaluated the effect of the *Brassica yellows* virus movement protein on anthocyanin concentrations in plants [51]. Chen demonstrated that the viral movement protein increases anthocyanin accumulation, resulting in the development of purple leaf symptoms in Arabidopsis thaliana. This study sheds light on how viral proteins influence plant metabolic pathways, particularly those involved in anthocyanin production, and how this links to the obvious signs of viral infection [51].

According to Linga R. Gutha, the symptoms of grapevine leafroll disease in red-fruited wine grape *(Vitis vinifera L.)* cultivars are green veins and crimson to reddish-purple discoloration in the interveinal portions of the leaves [49]. He proposed that the reddish-purple hue observed in symptomatic leaves was due to anthocyanin accumulation, which might be caused by up-regulation of genes involved in their biosynthesis. This concept highlights the importance of anthocyanins as a potential indicator of grapevine leafroll disease symptoms and proposes a genetic response to viral infection [49].

Conclusion

In this work, it was found that TBSV viral infection leads to an increase in the level of hydrogen peroxide accumulation and also contributes to the accumulation of anthocyanins in TBSV-infected N. benthamiana plants. As is known, hydrogen peroxide and anthocyanin play an indispensable role in the implementation of various mechanisms of plant resistance to pathogens and other adverse environmental factors. The results presented in this work allow us to better understand the action of plant defense mechanisms and can be used to develop new methods for increasing the resistance of crops to viral infections.

Author Contributions

M.B., A.S., and **A.K.** – conceptualization; **M.B.** and **A.S.** – data curation; **M.B., A.S.,** and **A.K. –** formal analysis; **M.K., A.B., N.I.,** and **S.Zh.** – investigation; **M.K., A.K., A.S.,** and **Zh.M.** – methodology; **M.B.** – visualization; **M.B., A.S.,** and **A.K.** - writing – original draft; **M.B., A.S.**, **A.K., S.Zh., A.B., N.I.,** and **Zh.M. –** writing – review & editing; **S.Zh., A.B., N.I.**, and **Zh.M**. – project administration; **M.B., A.K.,** and **Zh.M.** – supervision**; Zh.M** - funding acquisition and resources. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Compliance with ethical standards

This article does not contain a description of studies performed by the authors involving people or using animals as objects.

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Өсімдіктің патогенге төзімділігіндегі антоцианиндердің рөлі

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Аңдатпа. Вирустық инфекциялар Қазақстанда және бүкіл әлемде ауыл шаруашылығы дақылдарының өсуіне, дамуына және өнімділігіне теріс әсер етіп, өсімдік шаруашылығына үлкен қауіп төндіреді. Құрғақшылық, экстремалды температура, топырақтың тұздануы және ауру қоздырғыштарының инвазиясы сияқты көптеген стресс жағдайында вирустар өсімдіктердің метаболизм жолдарын бұзу және қолайсыз факторларға төзімділігін төмендету арқылы физиологиялық өзгерістерді күшейтеді. Өсімдіктердің қорғаныс механизмдеріне қатысатын маңызды қосылыстардың антоцианиндердің биосинтезіне вирустық инфекциялардың әсеріне ерекше назар аударылады. Бұл жұмыста қызанақтың бұталы ергежейлігінің вирусын (TBSV) зақымдау үлгісі өсімдіктің ортаңғы жапырақтарын басқа жапырақтармен салыстырғанда көбірек зақымдайтыны, ал үстіңгі жапырақтардың өспейтіндігі көрсетілген. Сонымен қатар, ортаңғы жапырақтарда некроз байқалады, бұл одан әрі бағдарламаланған жасушалардың жойылуына

(ПКД) әкеледі. Сонымен қатар, TBSV инфекциясы *Nicotiana benthamiana* өсімдіктерінде сутегі асқын тотығы деңгейінің айтарлықтай жоғарылауына және антоцианиннің жиналуына әкеледі. Бұл өзгерістер тотығу-тотықсыздану тепе-теңдігінің бұзылуын және вирустық инфекцияға жауап ретінде қорғаныс реакцияларының белсендірілуін көрсетеді. Нәтижелер өсімдіктің вирустық инфекцияларға және басқа да стресс факторларына төзімділігін арттыру стратегияларын әзірлеу үшін вирус пен өсімдіктердің өзара әрекеттесуін зерттеудің маңыздылығын көрсетеді.
Түйін сөздер: *N. benthamiana*, вирус, TBSV, биотикалық стресс, ОБТ, H_2O_2, антоцианин

Роль антоцианов в устойчивости растений к патогену

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Аннотация. Вирусные инфекции представляют серьёзную угрозу для растениеводства в Казахстане и во всём мире, оказывая негативное влияние на рост, развитие и продуктивность сельскохозяйственных культур. В условиях множественных стрессов, таких, как засуха, экстремальные температуры, засоление почв и поражение патогенами, вирусы усугубляют физиологические изменения в растениях, нарушая их метаболические пути и снижая устойчивость к неблагоприятным факторам. Особое внимание уделяется влиянию вирусных инфекций на биосинтез антоцианов – важных соединений, участвующих в защитных механизмах растений. В данной работе показано, что при заражении вирусом кустистой карликовости томатов (TBSV) средние листья модельного растения повреждаются сильнее по сравнению с другими листьями, а верхние листья остановились в развитии. Кроме того, в средних листьях наблюдается некроз, что приводит к дальнейшему программируемому клеточному разрушению (PCD). Более того, инфекция вирусом TBSV приводит к значительному повышению уровня перекиси водорода и накоплению антоцианов в растениях *Nicotiana benthamiana.* Эти изменения свидетельствуют о нарушении окислительно-восстановительного баланса и активации защитных реакций в ответ на вирусную инфекцию. Полученные данные подчеркивают важность изучения взаимодействия вирусов с растениями для разработки стратегий повышения устойчивости сельскохозяйственных культур к вирусным инфекциям и другим стрессовым факторам.
Ключевые слова: *N. benthamiana,* вирус, TBSV, биотический стресс, АФК, H_2O_2, антоциан

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Research article

Endogenous purines as natural ligands of the A_{2B} adenosine receptor

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Abstract. Endogenous purines are essential regulators of various physiological functions, including immune response, inflammation, and neurotransmission. While adenosine has long been considered the primary ligand for adenosine receptors, recent evidence suggests that other purines may also interact with these receptors, particularly the A_{2B} adenosine receptor (A_{2B}AR). This study investigates the potential role of endogenous purines as natural ligands of A_{2B}AR using molecular docking. The results demonstrate a high binding affinity of purines for A_{2B}AR, suggesting their functional relevance in receptor-mediated signaling. Additionally, A_{2B}AR plays a crucial role in immune regulation by influencing T-cell differentiation and cytokine production. Modulating its activity through endogenous purines may have significant implications for inflammation-related diseases, including cancer and neurodegenerative disorders. The findings provide new insights into the purinergic control of the adenosinergic system and highlight the potential of targeting A2BAR in therapeutic strategies. However, further studies, including *in vitro* and *in vivo* experiments, are necessary to confirm the physiological relevance of these interactions. This research expands our understanding of purinergic signaling and opens new avenues for the development of pharmacological interventions aimed at modulating immune and inflammatory responses.

Keywords: A_{2B} adenosine receptor, purinergic signaling, immune modu-lation, inflammation and neurodegeneration, molecular docking analysis

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Introduction

Adenosine receptors (ARs) are G protein-coupled receptors that play crucial roles in various physiological processes, including inflammation, cardiovascular function, and neurotransmission [1, 2]. The four AR subtypes (A_1, A_{2A}, A_{2B}, and A_3) have unique pharmacological profiles and tissue distributions, making them promising therapeutic targets for numerous diseases [2, 3]. ARs modulate neurotransmission in the central nervous system, interfering with dopaminergic, glutamatergic, and other neurotransmitter systems, which suggests potential applications in treating neuropsychiatric disorders [4]. Recent advancements in AR pharmacology have led to the development of new ligands and strategies for receptor activation, showing promise in treating conditions such as cancer, inflammatory diseases, and metabolic disorders [3]. However, the ubiquitous distribution of ARs presents challenges in achieving selective and site-specific modulation [1, 3]. Significantly, the A_{2B} adenosine receptor (A_{2B}AR) is a low-affinity receptor with wide distribution, making it a target of interest in various pathological conditions [5].

The A_{2B}AR has become a significant target in various pathological conditions, including inflammatory diseases, cardiovascular disorders, and metabolic dysfunctions [6,7]. A_{2B}AR activation plays a crucial role in alcoholic hepatitis by regulating cAMP levels and the NF-κB pathway, potentially reducing inflammation and steatosis [8]. While A2BAR antagonists have shown promise in treating airway inflammation, gastrointestinal disorders, and cancer, only a few have entered clinical trials [6]. Recent research has focused on developing allosteric modulators for A_{2B}AR, which offer advantages over orthosteric ligands by fine-tuning tissue responses to endogenous agonists [5]. These modulators may prove beneficial in managing chronic obstructive pulmonary disease, protecting the heart from ischemic injury, and promoting bone formation [5]. Overall, A_{2B}AR-targeted therapies represent a promising avenue for treating various human diseases.

Interestingly, initially A_{2B}AR was considered less physiologically relevant due to its low affinity for adenosine, has gained attention for its upregulation during hypoxia and inflammation [9,10]. Studies using genetic and pharmacological approaches have demonstrated A_{2B}AR's tissue-protective role in various acute disease models [10, 11]. Additionally, the adenosine metabolite inosine has been identified as a functional agonist of the related A_{2A} receptor, exhibiting a unique signaling bias compared to adenosine [12]. This finding suggests that inosine may play a role in prolonging adenosine receptor activation in vivo, given its longer half-life. These advances provide new insights into purinergic signaling and potential other purines, such as inosine, guanosine, and their metabolites, which may contribute to A_{2B}AR modulation. These alternative ligands could influence receptor signaling, offering new insights into the physiological and pathological roles of the A_{2B}AR receptor.

An understanding of the interaction between endogenous purines and A_{2B}AR could provide valuable information for the development of pharmaceuticals. While synthetic compounds targeting A_{2B}AR have shown promise in preclinical and early clinical studies, the presence of natural ligands raises important questions about receptor selectivity, competitive binding, and functional outcomes [13-15]. Some endogenous purines have been suggested to exert

protective effects in ischemic injury, neuroinflammation, and metabolic regulation, indicating their potential as modulators of A_{2B}AR activity [16, 17].

This study investigates endogenous purines as natural ligands of A_{2B}AR and their influence on receptor signaling pathways using an AlphaFold-modeled receptor and molecular docking. This approach enables a detailed analysis of ligand-binding characteristics and their potential impact on receptor signaling mechanisms. A deeper understanding of these interactions may reveal new aspects of A_{2B}AR pharmacology and regulation and provide insights into the potential use of purines as biomarkers or therapeutic targets in diseases associated with A_{2B}AR dysfunction.

Materials and research methods

3D Model of the Adenosine Receptor

3D structures of the active and inactive conformations of the adenosine receptor type A_{2B} (A_{2B}AR) were obtained by the program AlphaFold2-MultiState AI (https://gpcrdb.org/structure/homology_models) [18,19].

Ligand Molecules

Based on structural similarities, it was hypothesized that all-natural purine nucleosides and purines could interact with the target proteins during the ligand library compilation for molecular docking [20]. Analysis of databases and literature led to the identification of two antagonists and one selective agonist that were used in this study.

Adenosine ($C_{10}H_{13}N_5O_4$) is a nucleoside composed of an adenine base linked to D-ribose, playing a key role in nucleic acid structure, energy metabolism, and cell signaling (PubChem CID: 60961) [21]. Inosine ($C_{10}H_{12}N_4O_5$) is structurally similar, participating in metabolic pathways and nitrogen compound exchange (PubChem CID: 135398641) [22]. Xanthosine ($C_{10}H_{12}N_4O_6$) serves as a metabolic intermediate (PubChem CID: 164959), while guanosine ($C_{10}H_{13}N_5O_5$) is crucial for genetic information transfer and cellular regulation (PubChem CID: 135398634) [23, 24].

Adenine ($C_5H_5N_5$) is a fundamental purine base in DNA, RNA, and ATP, essential for energy transfer (PubChem CID: 190) [25]. Hypoxanthine ($C_5H_4N_4O$) is an intermediate in purine metabolism (PubChem CID: 135398638), while xanthine ($C_5H_4N_4O_2$) is a precursor to biologically active compounds (PubChem CID: 1188) [26–29]. Guanine ($C_5H_5N_5O$) is a key nucleobase involved in genetic coding and cellular processes (PubChem CID: 135398635) [30].

Caffeine ($C_8H_{10}N_4O_2$) is a xanthine-derived alkaloid that acts as a non-selective A2BAR antagonist, enhancing wakefulness and cognitive function (PubChem CID: 2519) [31–33]. MCP-NECA ($C_{21}H_{20}N_8O_3$) is a potent A_{2B}AR antagonist with potential applications in pain relief and neurodegenerative disease treatment (PubChem CID: 5310960) [34]. BAY 60-6583 ($C_{19}H_{17}N_5O_2S$) is a selective A_{2B}AR agonist with strong affinity, used to investigate A_{2B}-mediated pathways in inflammation, cardiovascular diseases, and cancer (PubChem CID: 135398635) [35]. These compounds provide valuable tools for exploring purinergic signaling and potential therapeutic applications.

Hardware and software

Molecular docking was carried out on a homemade computer, the system includes the motherboard ASRock Super Alloy B250 Pro4, video cards MSI NVIDIA GeForce GTX 1050 Ti 4Gb, Gigabyte NVIDIA GeForce GTX 1050 Ti 4Gb, processor Intel Pentium G4600, RAM Kingston Fury Beast KF432C16BBK2/16 16, permanent memory SSD Kingston SA400S37 480 Gb. To perform rigid molecular docking, we used the AutoDock Vina GPU program [36-38]. AutoDock is a program developed for molecular docking. It is mainly used for protein-ligand docking, including taking into account mobile protein residues. However, during the study, difficulties were identified in working on AutoDock Vina running on the CPU, and AutoDock Vina GPU, which runs on Linux, was adapted to work on Windows. The program itself was downloaded from open access on the GitHub website (https://github.com/DeltaGroupNJUPT/Vina-GPU-2.0).

Rigid Docking Using AutoDock

To perform molecular interaction, we used Rigid docking, which assumes the stability of the molecule and a specific binding site. The binding site was determined by the reference structure of the A_{2B}AR in AutoDock, and a Grid Box was also built on this basis with coordinates [39]. Also, a script with a configuration using the Grid Box coordinates was written to perform docking. The protein was also prepared in AutoDock, with the water removed from the structure, polar hydrogens added, and the charge calculated using Gasteiger [39,40]. The ligand molecule was also prepared in the same way after the structure was converted from SDF to PDB. Docking was performed with ligand and protein files in PBDQT format after preparation in AutoDock [36–38].

Visualization and Statistical Analysis

Molecular docking results were visualized and analyzed using PyMOL [41]. Structural alignments, ligand-receptor interactions, and binding poses were examined to identify key interactions contributing to ligand affinity. Statistical validation of docking results was performed by conducting docking simulations on five independent models of A_{2B}AR to ensure reproducibility [42]. Meaning energies and standard deviations were calculated to assess the stability of predicted binding interactions. This approach enabled a comprehensive comparison between rigid and flexible docking methods, providing a detailed understanding of ligand binding behavior in both active and inactive states of A_{2B}AR.

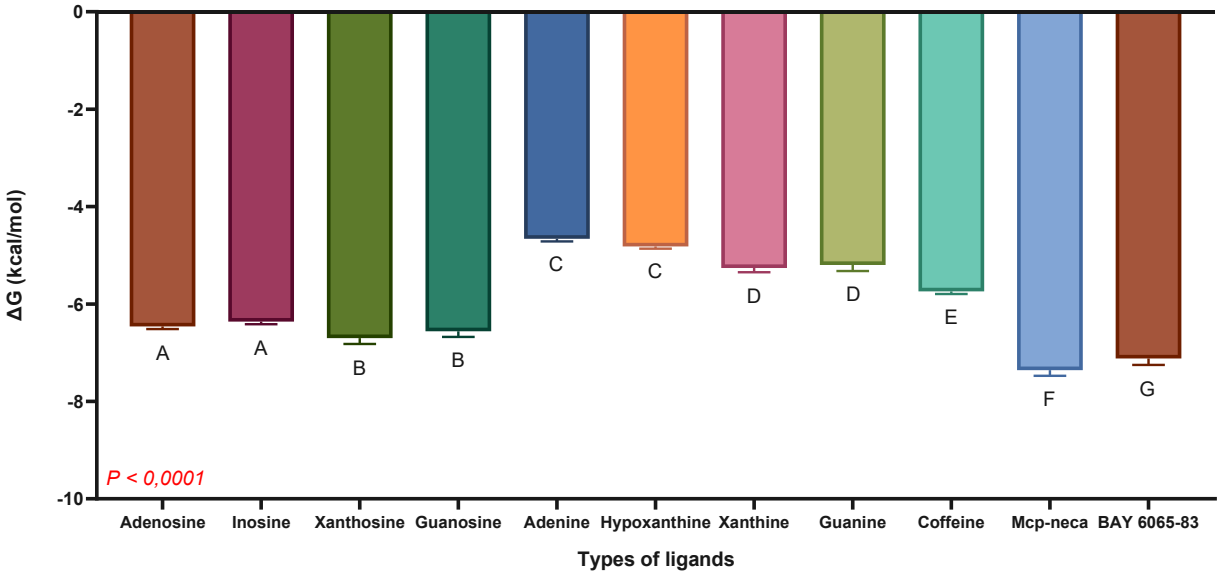
Results

In silico analysis of nucleotide binding to the inactive A_{2B}AR

In this study, molecular docking of 11 ligands with the inactive form of the A_{2B} adenosine receptor (A_{2B}AR) was performed to identify key interactions (Figure 1). The affinity of the compounds and their ability to bind to the receptor's active site were evaluated. The obtained data allowed for the determination of structural features influencing ligand interactions with A_{2B}AR. Statistical analysis was conducted using one-way analysis of variance (ANOVA) with a significance level of $P < 0.0001$, followed by Tukey's multiple comparisons test to assess differences between the ligands and the control group. The data confirms statistically significant differences in the affinity of the compounds studied for A_{2B}AR.

Adenosine was considered both a control and an experimental molecule, as its interaction with A_{2B}AR in the cryo-EM structure has already been described [21, 43]. Initially, an analysis

of adenosine binding revealed 16 possible binding variations, driven by different ligand conformations. The binding free energy (ΔG) analysis showed a minimum value of −5.8 kcal/mol and a maximum of −6.5 kcal/mol. The results indicate that adenosine binds to A2BAR in its inactive state through interactions with residues ASN254, GLU174, PHE173, HIS280, and ALA82. ASN254 is localized in the α6-helix, GLU174 and PHE173 in the α5-helix, ALA82 in the α3-helix, and HIS280 in the α7-helix (Figure 2A). The mobility of these helices, as demonstrated by TM analysis, is necessary for conformational changes and the release of the Gs protein subunit [19].



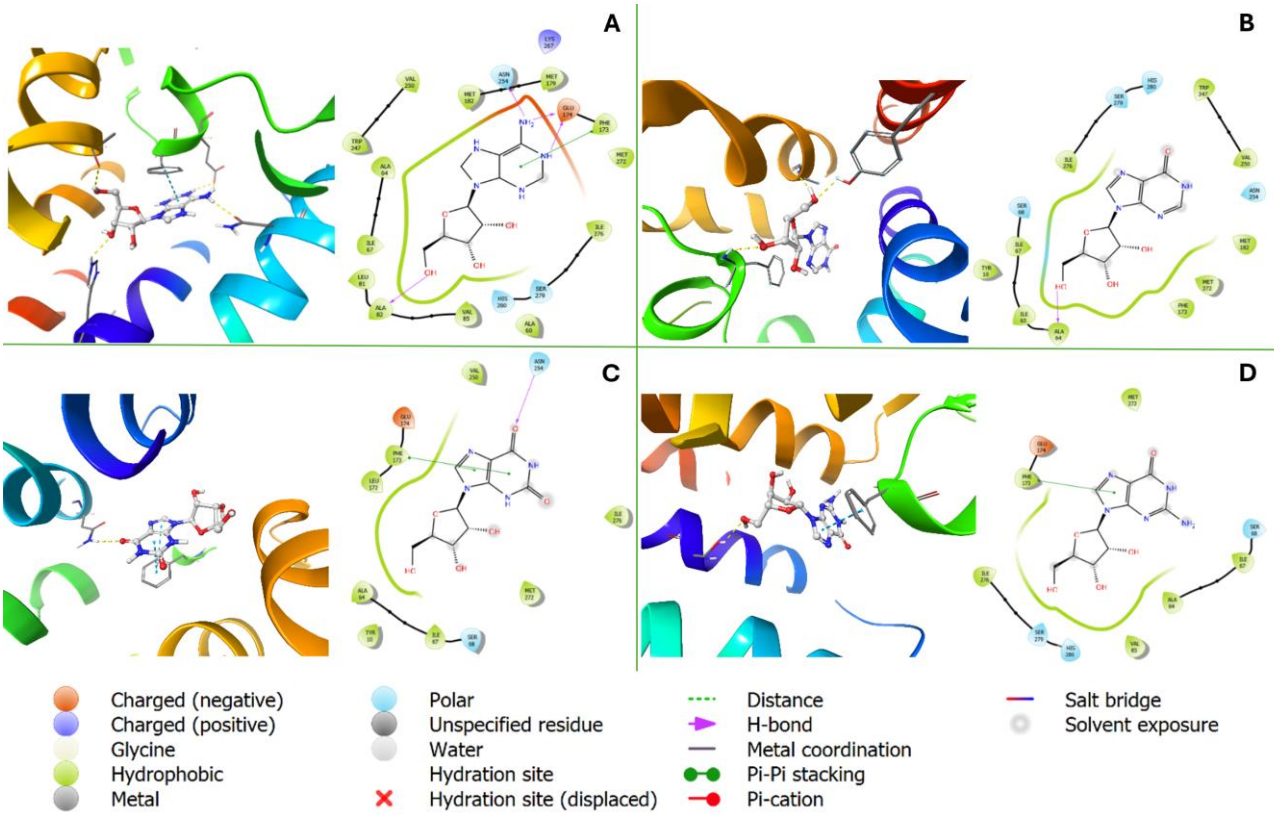
Note: Columns labeled with the same letters do not show statistically significant differences, whereas different letters indicate statistically significant differences.

Figure 1. Changes in ΔG upon ligand binding to the inactive A_{2B}AR

A hydrogen bond is formed between the amino group of adenosines at the 6-position of the purine ring and the carboxamide group of ASN254. The secondary amino group of adenosines at the 1-position of the purine ring may interact with the carboxyl group of GLU174. PHE173 participates in a π-π interaction between its benzene ring and the pyrimidine ring of the ligand. The hydroxyl group at the 5'-position of ribose forms a hydrogen bond with ALA82, while HIS280 interacts with the hydroxyl group of ribose at the 4'-position. These interactions have also been described in a study on the active structure of A_{2B}AR obtained by cryo-EM, further demonstrating that docking with AlphaFold-predicted structures is relevant for conducting molecular simulations [19,43].

Inosine was selected for docking due to its structural similarity to adenosine, with the only difference being the substitution of the amino group at the 6-position of the purine ring with an oxygen atom [22]. The maximum binding affinity of inosine (ΔG = -6.5 kcal/mol) was found to be comparable to that of adenosine. However, unlike adenosine, inosine interacts with A_{2B}AR exclusively through its ribose moiety, without involving the purine ring. The primary

interactions occur with residues ALA64, PHE173, and TYR10, located in the α2-, α5-, and α1-helices, respectively (Figure 2B). Inosine forms hydrogen bonds between the hydroxyl groups of its ribose moiety and the amide bond of the polypeptide chain at PHE173, as well as with the hydroxyl group of TYR10. These findings suggest a potential role for inosine as a physiological antagonist of A_{2B}AR.



Note: α1 - red; α2 - orange; α3 - ochre-yellow; α4 - lemon; α5 - green; α6 - turquoise; α7 - blue.

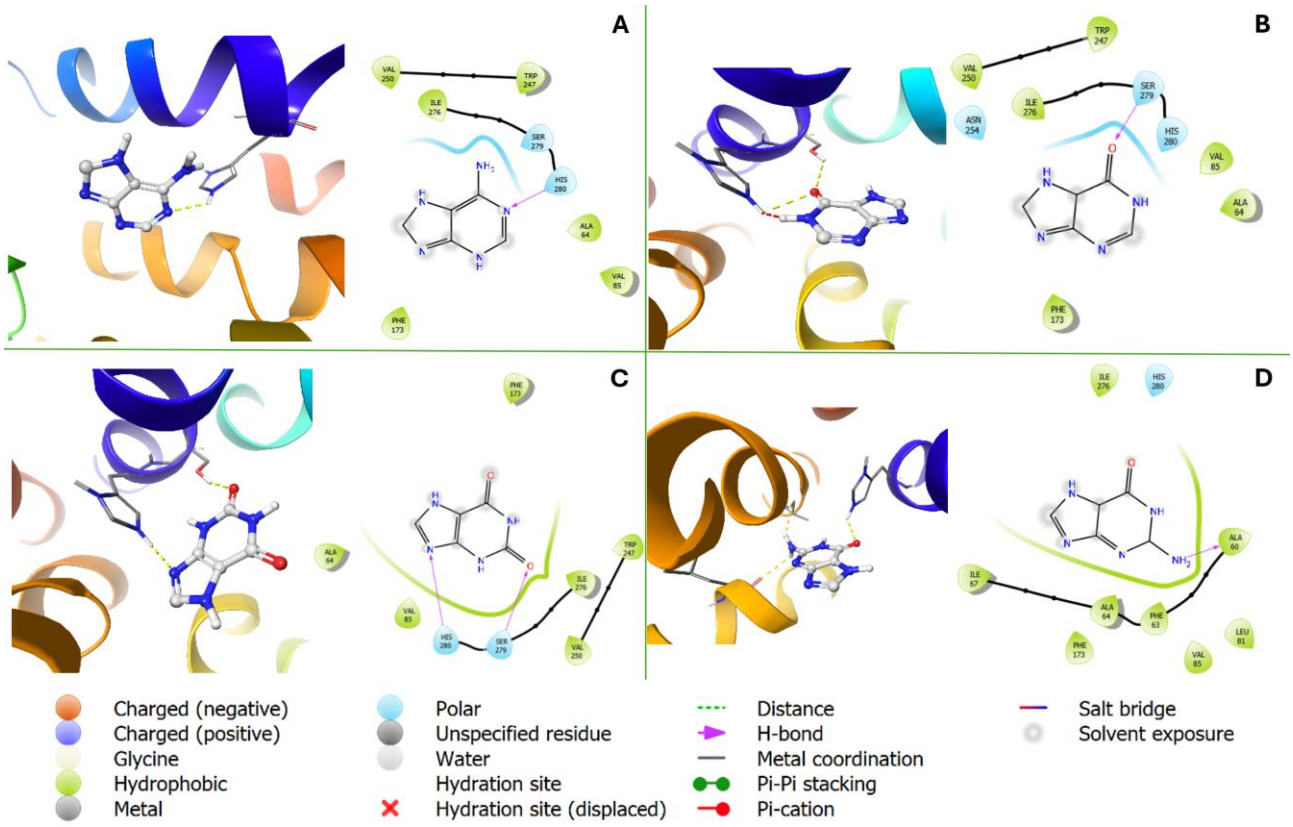
Figure 2. 3D and 2D visualization of molecular interactions between **(A)** Adenosine, **(B)** Inosine, **(C)** Xanthosine, and **(D)** Guanosine and the binding site of the inactive A_{2B}AR

Xanthosine, a less common purine nucleoside, was tested to assess its binding capacity to the inactive form of A_{2B}AR. Docking analysis revealed that xanthosine exhibits a higher binding affinity (ΔG = -6.9 kcal/mol) compared to adenosine and inosine. The structural distinction of xanthosine lies in the presence of an additional oxygen atom at the 2-position of the purine ring, which may contribute to stronger receptor binding.

The most stable conformation of xanthosine interacts with PHE173 and ASN254, located in the α5- and α6-helices, respectively (Figure 2C). PHE173 forms a double π-π interaction with the purine ring of the ligand, whereas adenosine exhibited only a single interaction. ASN254 forms a hydrogen bond between its carboxamide amino group and the oxygen at the 6-position of the purine ring. The absence of interaction with GLU174 and the altered position of the secondary amino group may indicate a potential agonistic activity of xanthosine toward A_{2B}AR.

Guanosine, the fourth tested nucleoside, is structurally similar to inosine but differs by the presence of an amino group at the 2-position of the purine ring. Docking analysis revealed that guanosine exhibits a higher binding affinity (ΔG = -6.7 kcal/mol) compared to adenosine. The primary interactions of guanosine with the receptor occur through residues PHE173 (α5-helix) and SER279 (α7-helix). PHE173 forms a π-π interaction with the benzene ring of the ligand, while SER279 participates in hydrogen bond formation with the hydroxyl group of the ribose at the 5'-position (Figure 2D). These findings suggest a potential role for guanosine as a physiological antagonist of A_{2B}AR.

Adenine is a derivative of adenosine lacking the ribose moiety. Molecular docking results indicated that its affinity for A_{2B}AR is lower than that of adenosine. The maximum and minimum binding free energies were ΔG = -4.7 and ΔG = -4.3 kcal/mol, respectively. Visualization of interactions demonstrated that adenine forms a single hydrogen bond between the tertiary amino group at the 1-position of the purine ring and the secondary amino group of HIS280 (Figure 3A). This amino acid is located on the α7-helix, which could potentially influence the conformation of the α8- and α1-helices. However, the lack of additional interactions suggests that adenine does not exert a significant effect on A_{2B}AR as either an agonist or an antagonist.



Note: α1 - red; α2 - orange; α3 - ochre-yellow; α4 - lemon; α5 - green; α6 - turquoise; α7 - blue.

Figure 3. 3D and 2D visualization of molecular interactions between **(A)** Adenine; **(B)** Hypoxanthine; **(C)** Xanthine; and **(D)** Guanine and the binding site of the inactive A_{2B}AR

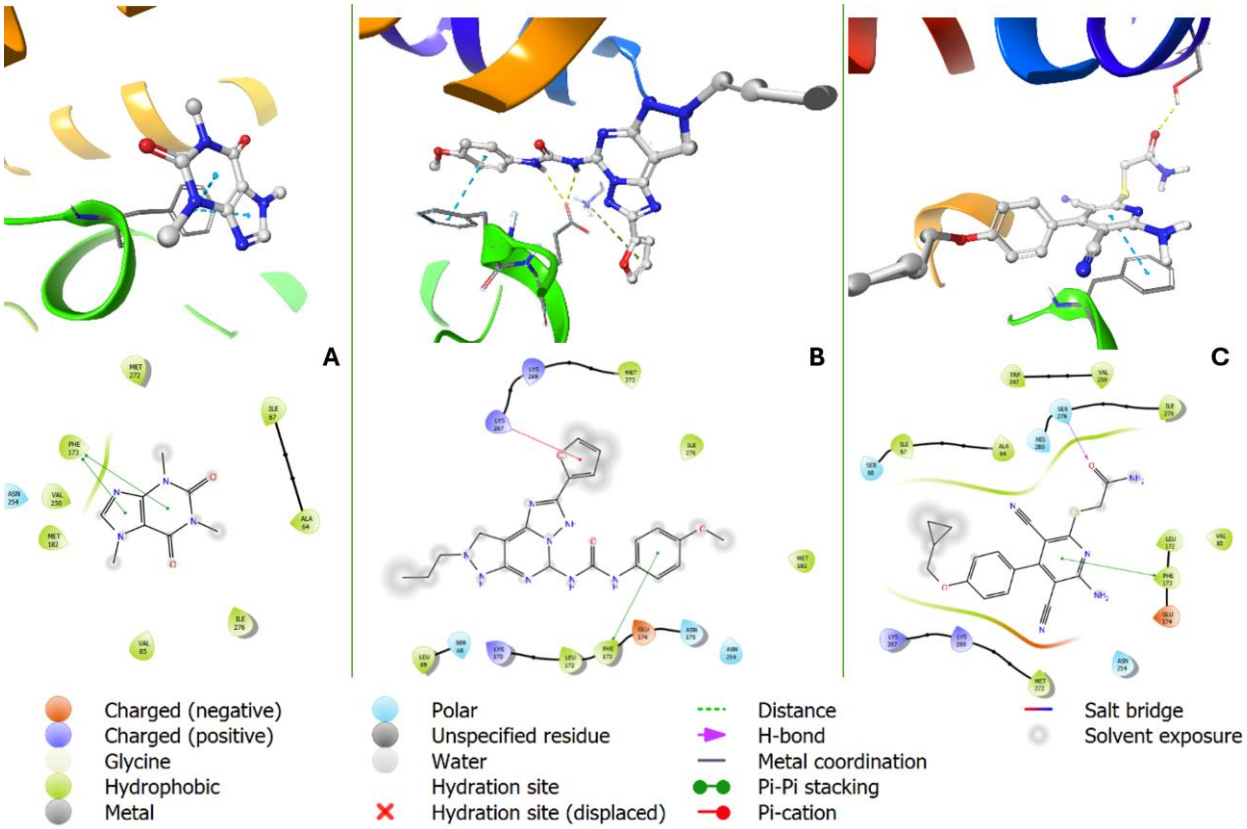
Hypoxanthine has been theoretically considered as a potential A2BAR antagonist, providing the rationale for its molecular docking analysis [44]. However, literature data indicate low receptor affinity for alloxazine under physiological conditions in vitro and in vivo. The obtained results demonstrated that hypoxanthine exhibits a lower affinity for A_{2B}AR compared to adenosine, with the best binding free energy recorded at ΔG = -4.9 kcal/mol. 2D and 3D visualizations revealed that the molecule forms two hydrogen bonds with the α7-helix of the receptor via HIS280 and SER279 (Figure 3B). Hypoxanthine interacts with HIS280 through the secondary amino group at the 1-position of the purine ring, while SER279 forms a hydrogen bond with its carbonyl group at the 6-position. These interactions suggest that binding to the α7-helix may influence the α1-helix, altering the binding pocket and potentially inhibiting the receptor.

Xanthine, a derivative of hypoxanthine, has been considered a potential antagonist of adenosine receptors. However, existing data suggests its possible role as an A_{2B}AR agonist. The primary structural difference between xanthine and hypoxanthine lies in the presence of an additional oxygen atom at the 2-position of the purine ring. Docking results revealed that xanthine exhibits a higher affinity for the receptor compared to hypoxanthine, with the best binding free energy recorded at ΔG = -5.4 kcal/mol. Visualization showed that xanthine forms similar interactions with HIS280 and SER279 but with additional functional contributions from the oxygen at the 2-position and the tertiary amino group at the 9-position (Figure 3C).

Guanine is the last of the naturally occurring purine bases circulating in the human body [30]. It differs from xanthine by the substitution of an oxygen atom at the 2-position of the purine ring with a primary amino group. Molecular docking revealed a similar binding free energy (ΔG = -5.4 kcal/mol), indicating its high affinity for A_{2B}AR. Visualization demonstrated that guanine interacts with three amino acid residues located in different receptor domains: LEU81 (α3-helix), ALA60 (α2-helix), and HIS280 (α7-helix). Hydrogen bonds are formed between LEU81 and the primary amino group at the 2-position of the purine ring, between ALA60 and the same group, as well as between HIS280 and the oxygen at the 6-position of the purine ring (Figure 3D). Potential conformational changes in these structural elements may lead to a reduction in the binding pocket size, making guanine a promising low-affinity antagonist of A_{2B}AR.

Caffeine is a well-known non-selective antagonist of adenosine receptors [31-33]. Numerous antagonists with specificity for different adenosine receptor subtypes have been synthesized based on their molecular structure. In this study, caffeine was used as a reference molecule to assess the inhibitory potential of the purine ring concerning the inactive form of the A_{2B}AR adenosine receptor.

Molecular docking analysis revealed two possible conformations of caffeine that could interact with A_{2B}AR in its inactive state (Figure 4A). Further molecular interaction analysis identified a key π-π interaction between caffeine and the PHE173 residue, which is known to play a significant role in receptor activation. Given the importance of this residue in receptor function, this interaction suggests that caffeine may act as a competitive inhibitor by interfering with ligand binding at the active site. These findings support the established role of caffeine as an effective modulator of adenosine receptor activity, further validating its function as a benchmark compound in receptor inhibition studies.



Note: α1 - red; α2 - orange; α3 - ochre-yellow; α4 - lemon; α5 - green; α6 - turquoise; α7 - blue.

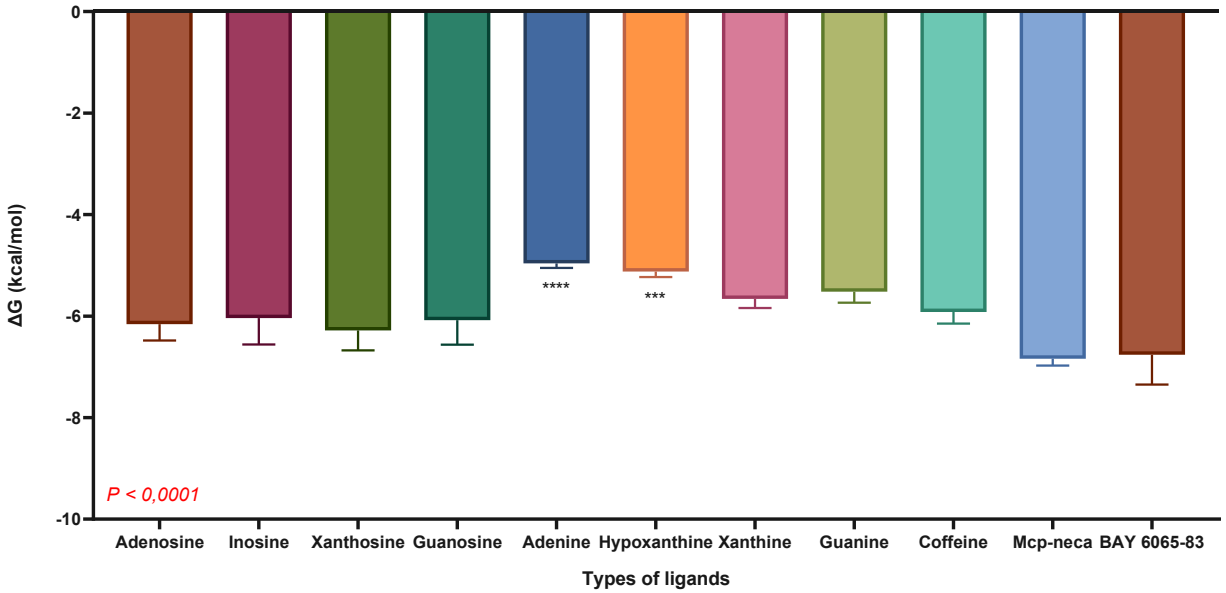
Figure 4. 3D and 2D visualization of molecular interactions of **(A)** Caffeine, **(B)** Mcp-neca, **(C)** BAY 6065–83 with the A_{2B}AR binding site

Mcp-neca, as previously described, is an antagonist of both A_{2B} and A3 AR [34]. In this study, molecular docking of Mcp-neca was performed with both inactive and active forms of A_{2B}AR to elucidate its inhibition mechanisms. The results indicate a significantly higher affinity of Mcp-neca compared to adenosine (ΔG = -7.5 kcal/mol, with a minimum value of ΔG = -6.8 kcal/mol). Interaction visualization (Figure 4B) revealed that Mcp-neca interacts with at least three amino acid residues: PHE 173, GLU 174, and LYS 267. The PHE 173 residue, located in the α5-helix, participates in a π-π interaction with the ligand's benzene ring, potentially stabilizing the complex. GLU 174 forms hydrogen bonds, which may restrict α5-helix mobility and prevent G-protein activation. The most significant contribution to affinity is likely to come from the π-cation interaction between the positively charged LYS 267 and the ligand's aromatic system. Thus, Mcp-neca acts as a competitive antagonist by blocking the active site and preventing adenosine

The final tested ligand was BAY 6065–83, known as a selective A_{2B}AR agonist. Analysis of cryo-EM structures identified active conformations of the receptor interacting with BAY 6065–83. Molecular docking results showed that the ligand's highest affinity was ΔG = -7.3 kcal/mol. Interaction visualization (Figure 4C) revealed two key interactions: a π-π interaction with PHE 173 (α5-helix) and a hydrogen bond with SER 279 (α7-helix). These interactions are characteristic of both adenosine receptor agonists and antagonists. Thus, BAY 6065–83 exhibits specific interactions with A_{2B}AR that may play a crucial role in its activation.

In silico analysis of nucleotide binding to the active $A_{2B}AR$

After molecular docking with the inactive form of the A2BAR receptor, similar calculations were performed for its active form. The study covered 11 different ligands, including natural purines as well as selective and non-selective antagonists and agonists (Figure 5). The analysis identified potential agonists (xanthosine), antagonists (inosine, guanosine, guanine), and molecules with no significant effect (adenine). The roles of xanthine and hypoxanthine remain uncertain.

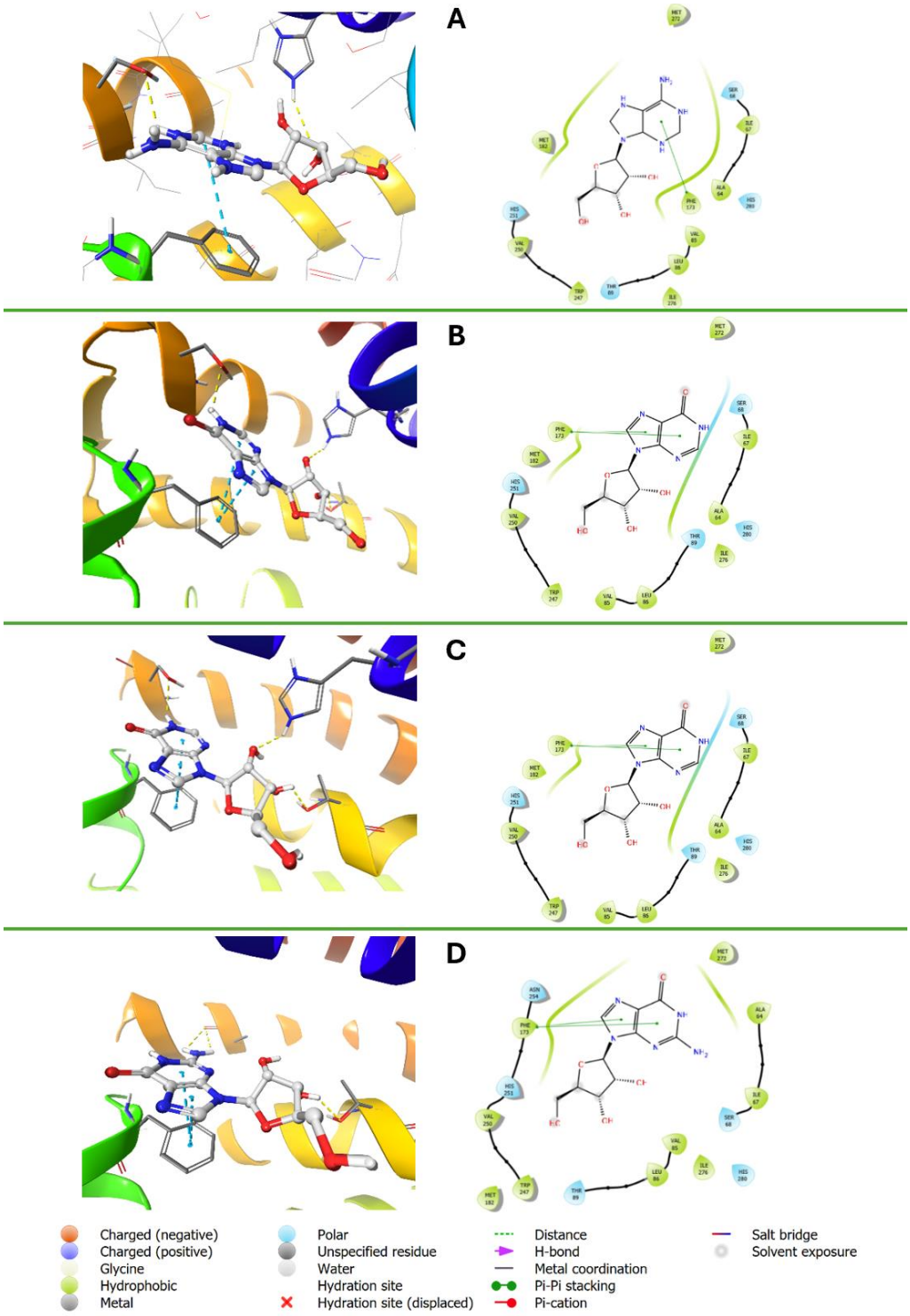


Note: Tukey's multiple comparison test was used to assess statistical significance relative to adenosine. Columns without labels are not significant, whereas asterisks indicate significance.

Figure 5. Change ΔG for ligand binding to the active $A_{2B}AR$

This section focuses on the analysis of natural nucleosides to confirm their functional role, as well as the selective antagonist MCP-NECA and the selective agonist BAY 6065–83. Affinity evaluation is based on binding free energy, analyzed only for the most stable complexes. Additionally, the interaction patterns of these ligands with key receptor residues were examined to provide further insights into their binding mechanisms.

The ΔG values for interactions between the active form of $A_{2B}AR$ and adenosine, inosine, xanthosine, and guanosine did not show significant differences in affinity (Figure 5). Among them, adenosine exhibited the lowest affinity (ΔG = -6.6 kcal/mol), while guanosine demonstrated the highest binding affinity (ΔG = -6.9 kcal/mol). The presumed agonist xanthosine displayed an intermediate ΔG value of -6.8 kcal/mol. Molecular interaction analysis revealed that all potential agonists form a π-π interaction with the hydrophobic residue PHE 173 (α5-helix). Adenosine, inosine, and xanthosine establish a hydrogen bond with HIS 280 (α7-helix) via the ribose moiety. Additionally, inosine, xanthosine, and guanosine interact with THR 89 (α2-helix), whereas guanosine, unlike the other molecules, forms a hydrogen bond with ALA 64 instead of SER 68 (Figure 6).



Note: α1 - red; α2 - orange; α3 - ochre-yellow; α4 - lemon; α5 - green; α6 - turquoise; α7 - blue.

Figure 6. 3D and 2D visualization of molecular interactions of **(A)** Adenosine, **(B)** Inosine, **(C)** Xanthosine, and **(D)** Guanosine with the binding site of the active $A_{2B}AR$

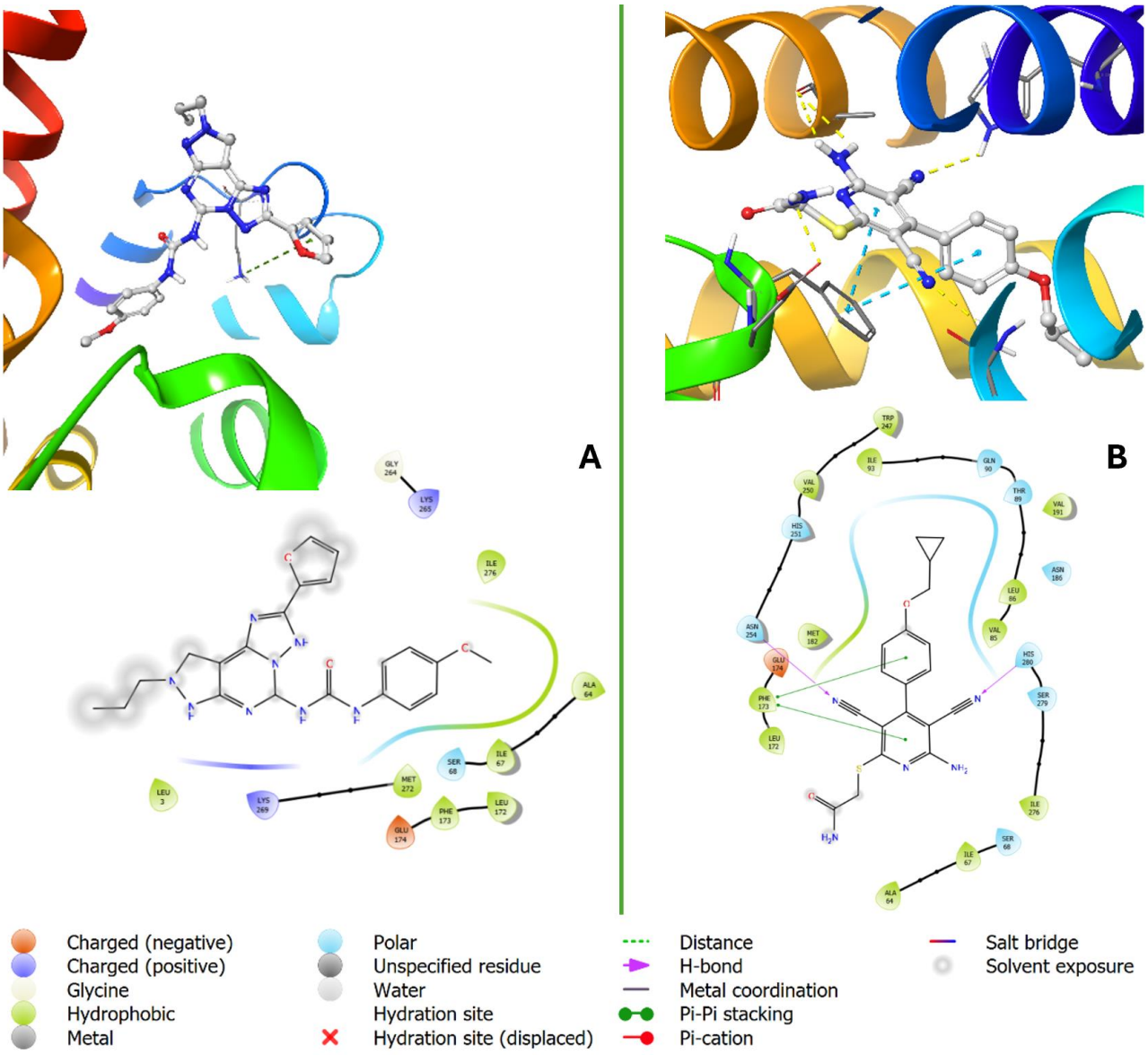
The data obtained suggest that xanthosine is likely to be an agonist of A_{2B}AR, as its interactions align with known parameters of agonistic activity. However, the influence of an additional hydrogen bond with THR 89 remains unclear and may merely enhance affinity without inducing conformational changes in the receptor. Inosine and guanosine also exhibit agonistic potential, but their interactions with key amino acids differ, which could indicate possible antagonistic activity of guanosine.

A literature review on the potential role of inosine in A_{2B}AR signaling revealed conflicting evidence. While several studies demonstrate that inosine interacts with A_{2A}AR adenosine receptors, these findings cannot be directly extrapolated to A_{2B}AR due to significant differences in their signaling mechanisms. A_{2A}AR primarily couples to Gs proteins, leading to increased intracellular cAMP levels, whereas A2BAR can couple to both Gs and Gq proteins, resulting in more complex and context-dependent signaling pathways. Studies on A_{2A}AR typically employ adenosine concentrations of 30 nM and 100 nM, whereas research on A2BAR indicates that this receptor has a relatively low affinity for its natural agonist, with a Ki value of 15,000 nM (equivalent to 15 μM) [12, 45, 46]. Interestingly, one study also utilized a significantly higher inosine concentration of 100 mM, which showed an increase in dynamic mass redistribution. However, this experiment was conducted on cells overexpressing A_{2A}AR, raising concerns about the validity of the observed effects [12]. Given the distinct functional roles and signaling pathways of A_{2A}AR and A_{2B}AR, it remains unclear whether inosine acts as an agonist, antagonist, or neutral ligand for A_{2B}AR. Direct functional assays are necessary to determine their precise activity.

These findings highlight the complexity of nucleoside-receptor interactions and suggest that further investigation is needed to determine the precise functional roles of inosine and guanosine in A_{2B}AR signaling. Future studies should aim to clarify whether their effects are context-dependent and whether variations in receptor expression levels influence their agonistic or antagonistic properties. It is important to note that in two separate experiments, the A_{2A} receptor antagonist [3H]ZM 241385 was used as a control agent to assess the binding of inosine to this receptor. However, this compound is also known to act as an A_{2B} antagonist, raising concerns regarding the validity of these findings. As a result, we cannot fully accept these data as definitive evidence [44]. Consequently, we propose the hypothesis that inosine may act as an A2BAR antagonist, but further experimental validation is required.

In this study, the selective antagonist Mcp-neca and the selective agonist BAY 6065–83 were analyzed in complex with the active form of A2BAR. The calculated binding free energy was ΔG = -7.4 kcal/mol for BAY 6065–83 and ΔG = -7.0 kcal/mol for Mcp-neca. Molecular docking results indicate that MCP-NECA loses a significant portion of its interactions in the active state of the receptor, although it retains π-π interactions with amino acid residues of the α7-helix.

In contrast, BAY 6065–83 exhibits interaction patterns similar to those of adenosine. Specifically, PHE 173 (α5-helix) forms a π-π interaction, while hydrogen bonds are established with HIS 280 (α7-helix), ALA 64 (α2-helix), GLU 174 (α5-helix), and ASN 254 (α5-helix). These findings suggest that BAY 6065–83 engages key structural elements involved in receptor activation, reinforcing its role as a potent A_{2B}AR agonist.



Note: α1 - red; α2 - orange; α3 - ochre-yellow; α4 - lemon; α5 - green; α6 - turquoise; α7 - blue.

Figure 7. 3D and 2D visualization of molecular interactions of **(A)** Mcp-neca, **(B)** BAY 6065–83 with the binding site of the active $A_{2B}AR$

Discussion

$A_{2B}AR$ plays a crucial role in various physiological processes, including inflammation, immune modulation, and vascular regulation [6-13]. Previously, adenosine and its analogs were considered to be the primary ligands for this receptor. However, our findings indicate that

all endogenous purines may also interact with A_{2B}AR. In this study, we have demonstrated that endogenous purines exhibit a high affinity for A_{2B}AR, as confirmed by molecular docking analysis. These results expand the current understanding of the role of purines in the regulation of the adenosinergic system and may have significant physiological and pharmacological implications.

Studies have shown that A_{2B}AR is involved in the differentiation of CD4+ T cells into regulatory T cells *(Tregs)* [47]. The work of Hiroko Nakatsukasa *et al.* demonstrated that A_{2B}AR antagonists inhibit Foxp3 expression and IL-10 production without affecting CD4+ T cell activation. These findings highlight the pivotal role of A_{2B}AR in immune regulation. Regulatory T cells play a crucial role in maintaining peripheral immune tolerance and suppressing excessive immune responses. Notably, a reduction in *Treg* numbers has been associated with enhanced antitumor immunity [47,48]. Consequently, A_{2B}AR antagonists, by inhibiting *Treg* differentiation, may enhance the effectiveness of immune responses against tumor cells.

In the study by Matthias Seifert et al., the influence of A_{2A} and A_{2B} adenosine receptors on murine CAR-T cells was investigated [49]. It was established that the non-specific adenosine receptor agonist NECA reduces the release of IFN-γ, IL-2, and TNF-α in a dose-dependent manner [49]. CAR-T cells predominantly express A2A and A2B AR; however, the potential expression of A1 and A3 AR cannot be ruled out. Given that the EC50 values for NECA are 14 nM (A1), 20 nM (A_{2A}), 2.4 μM (A_{2B}), and 6.2 nM (A_3), it can be inferred that the observed reduction in cytokine secretion may also be influenced by the activation of A_1 and A_3 receptors, rather than being exclusively attributed to A_{2A} and A_{2B} [49,50].

Literature data confirm that the primary immunosuppressive effect of adenosine in CAR-T cells is mediated through the A_{2A} receptor [51]. Consequently, its activation suppresses the production of pro-inflammatory cytokines, which may be linked not only to the Gαs protein but also to the involvement of Gβ/γ subunits. Experimental data indicate that at low concentrations of the A_{2A} antagonist AB928 (<10 nM, EC50 = 2 nM), the secretion of pro-inflammatory cytokines is also reduced [52]. This suggests that A2B signaling in CAR-T cells may activate the Gq pathway, promoting differentiation without directly affecting cytokine synthesis. In contrast, A_{2B} antagonists inhibit differentiation while having no significant impact on cytokine production.

Inflammation plays a crucial role in the development of neurodegenerative disorders such as Alzheimer's and Parkinson's disease. Neuroinflammation, characterized by elevated levels of pro-inflammatory cytokines and activated microglia, contributes to disease progression [53]. Chronic microglial activation leads to increased production of inflammatory mediators, forming a vicious cycle of neuronal damage and further inflammation [54]. Protein aggregates, common in neurodegenerative diseases, can both induce and exacerbate neuroinflammation [53]. In Parkinson's disease, immune alterations in response to extracellular α-synuclein may modulate disease progression [55]. While it remains unclear whether neuroinflammation is a primary cause or secondary consequence of neurodegeneration, targeting inflammatory processes has emerged as a promising therapeutic strategy [53,54]. The activation of adenosine receptors, particularly A_{2B}, may influence neuroinflammatory processes by modulating immune cell activity in the central nervous system. Our findings suggest a potential role for endogenous purines in regulating inflammation through A2B receptors, opening new avenues for exploring their involvement in the pathogenesis of neurodegenerative diseases.

Despite the significance of our results, this study has certain limitations. Our conclusions are currently based solely on molecular docking, which indicates potential interactions between endogenous purines and the A_{2B} adenosine receptor. However, additional biochemical studies, including experiments on cell lines and in vivo models, are necessary to confirm their physiological role.

If A_{2B} AR indeed influences T-cell differentiation, this could present new opportunities for immunotherapy. On one hand, activation of this receptor may promote the development of regulatory T cells (Treg), which are essential for controlling autoimmune diseases and inflammatory processes. On the other hand, the inhibition of A_{2B} AR could enhance anti-tumor immunity by suppressing Treg differentiation and increasing the activity of effector T cells. Future research should focus on elucidating the effects of endogenous purines on A_{2B} AR in the context of immune regulation, which could provide valuable insights into their potential therapeutic applications in cancer and inflammatory diseases.

Author Contributions

M.S. – conceptualization; **M.S.–** methodology; **M.S**.– investigation; **M.S.–** validation; **M.S.–** data curation; **M.S. –** visualization; **M.S. –** writing – original draft; **M.S.** and **E.Ch.** – writing – review & editing.

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Conflicts of Interest

The authors declare no conflict of interest.

Compliance with ethical standards

This article does not contain a description of studies performed by the authors involving people or using animals as objects.

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Эндогендік пуриндер A_{2B} аденозин рецепторының табиғи лигандтары ретінде

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Аңдатпа. Эндогендік пуриндер әртүрлі физиологиялық функциялардың, соның ішінде иммундық жауаптың, қабынудың және нейротрансмиссияның маңызды реттеушілері болып табылады. Аденозин ұзақ уақыт бойы аденозиндік рецепторлар үшін негізгі лиганд болып саналғанымен, соңғы деректер басқа пуриндердің де осы рецепторлармен, атап айтқанда A_{2B} аденозиндік рецепторларымен (A_{2B}AR) әрекеттесе алатынын көрсетеді. Бұл зерттеу молекулалық докинг арқылы A_{2B}AR табиғи лигандтары ретінде эндогендік пуриндердің әлеуетті рөлін зерттейді. Нәтижелер пуриндердің A_{2B}AR-ға жоғары жақындығын көрсетеді, бұл олардың осы рецептор арқылы болатын сигнал беру жолдарындағы функционалдық маңыздылығын көрсетеді. Сонымен қатар, A_{2B}AR Т жасушаларының дифференциациясына және цитокиндердің өндірісіне әсер ету арқылы иммундық реттеуде шешуші рөл атқарады. Оның белсенділігін эндогендік пуриндермен модуляциялау ісік және нейродегенеративті бұзылыстарды қоса, қабыну ауруларын емдеуге айтарлықтай әсер етуі мүмкін. Алынған деректер аденозинергиялық жүйенің пуринергиялық бақылауы туралы жаңа түсініктер береді және терапевтік мақсат ретінде A_{2B}AR әлеуетін көрсетеді. Дегенмен, осы өзара әрекеттесулердің физиологиялық маңыздылығын түпкілікті растау үшін *in vitro* және *in vivo* эксперименттерін қоса, қосымша зерттеулер қажет. Бұл зерттеу пуринергиялық сигнализация туралы түсінігімізді кеңейтеді және иммундық және қабыну реакциясын модуляциялауға бағытталған фармакологиялық араласуды дамыту үшін жаңа жолдарды ашады.

Түйін сөздер: A_{2B} аденозин рецепторы, пуринергиялық сигнализация, иммундық модуляция, қабыну және нейродегенерация, молекулалық докинг талдауы

Эндогенные пурины как естественные лиганды A_{2B} рецептора аденозина

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Аннотация. Эндогенные пурины являются важными регуляторами различных физиологических функций, включая иммунный ответ, воспаление и нейротрансмиссию. Хотя аденозин долгое время считался основным лигандом аденозиновых рецепторов, последние данные свидетельствуют о том, что другие пурины также могут взаимодействовать с этими рецепторами, в частности, с A_{2B}-аденозиновым рецептором (A_{2B}AR). В данном исследовании изучается потенциальная роль эндогенных пуринов как природных лигандов A_{2B}AR с использованием молекулярного докинга. Результаты демонстрируют высокую аффинность пуринов к A_{2B}AR, что указывает на их функциональную значимость в сигнальных путях, опосредованных этим рецептором. Кроме того, A_{2B}AR играет ключевую роль в иммунной регуляции, влияя на дифференцировку Т-клеток и продукцию цитокинов. Модуляция его активности эндогенными пуринами может иметь значительное значение для лечения воспалительных заболеваний, включая рак и нейродегенеративные расстройства. Полученные данные дают новые представления о пуринергическом контроле аденозинергической системы и подчеркивают перспективность A_{2B}AR как терапевтической мишени. Однако для окончательного подтверждения физиологической значимости этих взаимодействий необходимы дополнительные исследования, включая эксперименты *in vitro* и *in vivo.* Это исследование расширяет понимание пуринергической сигнализации и открывает новые возможности для разработки фармакологических вмешательств, направленных на модуляцию иммунного и воспалительного ответа.

Ключевые слова: A_{2B} рецептор аденозина, пуринергическая сигнализация, иммунная модуляция, воспаление и нейродегенерация, молекулярный стыковочный анализ

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