

Brief information about the project

Name of the project	IPH № AP26199175 "Research on stevia breeding lines for the creation of specialized beverages with improved functional properties "
Relevance	Currently, the prevalence of diabetes mellitus (DM) is reaching the proportions of a global epidemic. According to the International Diabetes Federation (IDF), the number of patients with DM in the world has reached 537 million, with a predicted growth to 783 million by 2045. In Kazakhstan, the situation is characterized by extremely negative dynamics: over the past 15 years, the number of registered cases has increased 3.5 times, reaching 439,327 people in 2022. In this regard, a priority for healthcare and the food industry is the search for safe natural sweeteners. Stevia (<i>Stevia rebaudiana</i>) is the most promising alternative to synthetic sweeteners, as its diterpenoid glycosides are 50–450 times sweeter than sucrose, have zero calories, and do not negatively affect the body. Despite favorable agro-climatic conditions in southern Kazakhstan for cultivating this crop, industrial production of Stevia in the region is absent. The main limiting factor is the shortage of varieties adapted to local soil and climatic conditions. Thus, the scientific relevance of the project lies in the need for a methodical justification of the selection process, the creation of highly productive lines with high adaptive potential, and the development of technologies for the production of domestic functional beverages based on them.
Purpose	To research and develop breeding lines of stevia with adaptive potential suitable for zoning in the Almaty region, and to create specialized beverages with enhanced functional properties.
Objectives	1. To research and develop breeding lines of stevia with adaptive potential suitable for zoning in the Almaty region. 2. To study the agrobiological and physiological-biochemical characteristics of breeding lines of stevia at various stages of ontogenesis. 3. Development of technology for specialized beverages with improved functional properties.
Expected and achieved results	Results Achieved in 2025 During the first stage of project implementation in 2025, in full compliance with the approved calendar plan, a comprehensive technology for developing Stevia selection lines was established using methods of plant physiology, cell selection, and clonal propagation. The technique for initiating <i>in vitro</i> culture was successfully refined, ensuring seed germination at a level of 80% (on 1/10 MS medium), and microclonal propagation with 100% rooting of apical buds on 1/2 MS medium. Through direct and step-wise selection, lines demonstrating tolerance to salt (NaCl) and osmotic (PEG) stress were obtained. The identified adaptive biochemical changes, specifically an

increase in proline content by 45.8–87.5% and sucrose content by up to 3 times, allowed for a reduction in the plant acclimatization period to 1.0–1.5 months. It was established that treating seeds with a 0.025% colchicine solution promotes positive morphophysiological changes: an increase in leaf area and thickness by 19% and 20% respectively, as well as a 50% enhancement in shoot branching.

During field trials at the Al-Farabi KazNU Agrobiological Station, the adaptation of *ex vitro* regenerants was carried out. Following the analysis of the vegetation period, the broad-leaved morphotype was identified as the most promising, demonstrating maximum branching intensity (10–15 shoots per plant) and high productivity indicators. Agrochemical monitoring confirmed the effectiveness of applying mineral fertilizers (N₁₂₀P₁₂₀K₁₂₀), which led to a 126% increase in mobile nitrogen content in the soil and a 55–60% increase in plant height.

Based on the stage results, an article was published in a first-quartile (Q1) scientific journal — *Engineered Science* (2025, Vol. 37, DOI: 10.30919/es1814), along with materials in the proceedings of the IX International Scientific and Practical Conference (Kharkiv) dedicated to the calculation of water-alcohol mixtures for obtaining Stevia extract.

Current Results for Q1 2026

During the 2026 reporting period, an in-depth laboratory analysis was conducted on the raw plant material of the broad-leaved Stevia genotype obtained through in vitro mutagenesis. Using the HPLC system (Shimadzu LC-40), a high accumulation of bioactive polyphenols and flavonoids was identified: Catechin (197.04 mg/L), Phloridzin (157.28 mg/L), Naringin (95.32 mg/L), and Epicatechin (32.96 mg/L). The analysis also confirmed the presence of target diterpene glycosides (stevioside and rebaudioside). A rich mineral profile with a predominance of Potassium (177.7 mg/g) and Calcium (98.2 mg/g) was established using atomic absorption spectroscopy. This genotype has been identified as the most promising source for the industrial production of functional beverages with antioxidant and hypoglycemic properties.

Based on the data obtained, a Republic of Kazakhstan patent application titled "Method for producing a functional isotonic beverage" (Registration No. 2026/0291.1 dated March 10, 2026) has been prepared and filed; it is currently undergoing formal examination. Scientific results are presented in the following prepared articles:

The paper "Application of Stevia extract in the production of isotonic beverages" (in Kazakh) has been accepted for publication in the journal Bulletin of Shakarim University (No. 1 (21) 2026, in press, scheduled for release in March 2026). The study of the physicochemical and organoleptic

	indicators of functional beverages has been submitted for peer review to the Bulletin of ATU. Simultaneously, a manuscript is being prepared for submission to an international peer-reviewed journal of the Q1/Q2 level. Expected Results for 2026 In accordance with the calendar plan, the completion of studies on the agrobiological and physiological-biochemical properties of the lines at various stages of ontogenesis is scheduled. A key result will be the preparation and filing of an invention patent application with the Eurasian Patent Organization (EAPO) to ensure international protection and inclusion in the <i>Derwent Innovations Index (Web of Science)</i> database. Publication activity in the current year includes the release of at least one article in a Scopus/WoS journal of Q1/Q2 level and two articles in journals recommended by the Committee for Quality Assurance in the Sphere of Science and Higher Education of the Republic of Kazakhstan (CQAES). Expected Results for 2027 At the final stage of the project (2027), in accordance with the provisions of the calendar plan, the following activities are scheduled: Development of industrial production technology and drafts of regulatory and technical documentation for specialized beverages with improved functional properties. Conducting a comprehensive assessment of the organoleptic and physicochemical characteristics, as well as safety indicators of the finished products. Publication of a monograph in a Kazakhstani publishing house, summarizing the research results for the entire period of the project's implementation. Fulfillment of final indicators for articles with a high impact factor (cumulatively for 2025-2027): publication of at least 3 (three) articles in peer-reviewed scientific journals (Q1, Q2, and/or Q3 according to the Web of Science database or having a CiteScore percentile in the Scopus database of at least 60). A mandatory requirement is the inclusion of at least one article in the "multidisciplinary" category. Publication of at least one article or review in a peer-reviewed foreign or domestic scientific publication recommended by the Committee for Quality Assurance in the Sphere of Education and Science (CQAES). Preparation and planned submission for the defense of a dissertation for the degree of Doctor of Philosophy (PhD) in the project profile within the timeframes stipulated by the competition regulations (including the possibility of defense within two years after the completion of the project).
Research team members with their identifiers (Scopus Author ID, Researcher ID, ORCID, if	1) Saltanat Asrandina, Scopus Author ID: 56230315800, Web of Science ResearcherID OXA-8765-2025 ORCID: https://orcid.org/0000-0003-1354-582X

available) and links to relevant profiles	2) Saule Atabayeva, Scopus ID: 56229597500 Web of Science ResearcherIDN-9656-2014 ORCID: https://orcid.org/0000-0002-4704-6909 3) Zhanar ChUNETOVA, Scopus ID: 57223450225 ResearcherID 57223450225 ORCID: https://orcid.org/0000-0002-1150-9668 4) Nurgul Mamytova, Scopus ID: 56136653200 Web of Science ResearcherIDJZT-8960-2024 ORCID: https://orcid.org/0000-0001-8605-0225 5) Yerlan Kirshibayev, Scopus author ID: 57224637517 https://orcid.org/0000-0002-6061-2039 6) Shoinbekova, Sabina Scopus ID: 8370741600 Web of Science ResearcherIDP-2773-2015 https://orcid.org/0000-0002-6922-3670 7) Kekibaeva, Anara K., Scopus ID: 56768127100 https://orcid.org/0000-0003-3639-1341 8) Ayazhan Koshybay, Web of Science ResearcherIDLNQ-7704-2024 https://orcid.org/0009-0004-8955-0072
Usage	The project results are multifaceted and can be applied in the following areas: Food Industry: implementation of the developed recipes for functional and isotonic beverages based on Stevia concentrates to expand the range of domestic dietary and specialized food products. Agriculture and Biotechnology: utilization of the developed selection lines with high adaptive potential for the regionalization and industrial cultivation of Stevia in the soil and climatic conditions of the Almaty region. Biopharmaceuticals: application of the obtained plant raw materials, enriched with bioactive polyphenols, flavonoids, and diterpene glycosides, as a basis for creating natural antioxidant and hypoglycemic agents. Educational Process: integration of the research findings into university curricula for "Biotechnology," "Biology," and "Food Safety" programs, as well as within the framework of PhD training.

Priority compared to real-world alternatives	Priority compared to real-world alternatives The scientific and practical value of the project results is characterized by the following advantages over existing analogues: Regional adaptability: for the first time in Kazakhstan, selection lines of Stevia have been obtained and studied that exhibit high tolerance to the specific conditions of the Almaty region (alkaline soils with pH 8.6, arid climate), ensuring their stable productivity in this region. Unique phytochemical profile: the studied target genotype surpasses standard samples in the content of key bioactive substances, including Catechin (197.04 mg/L) and Phloridzin (157.28 mg/L). This, combined with a high level of potassium (177.7 mg/g), provides the final product with enhanced nutraceutical value. Technological efficiency: the developed technology of <i>in vitro</i> mutagenesis and microclonal propagation allowed for the reduction of the plant acclimatization period from 2.5 to 1.0-1.5 months while maintaining 100% rooting efficiency, which significantly accelerates the process of obtaining commercial raw materials. Functional synergy: unlike traditional Stevia-based sweeteners, the beverages under development possess a proven complex action due to the synergy of diterpene glycosides and the flavonoid complex, providing simultaneous antioxidant and hypoglycemic effects.
List of publications with links to them	1) Saltanat Asrandina, Zhanar ChUNETOVA, Saule Atabayeva, Yerlan Kirshibayev, Saule Kenzhebayeva, Nurgul Mamytova, Sabina Shoinbekova «Study of morphogenetic and physiological responses of Stevia rebaudiana to colchicine treatment in an in vitro micropropagation system and ex vitro adaptation» // Engineered Science. – 2025. – Vol. 37. p.1105. – Article 1814. DOI: https://dx.doi.org/10.30919/es1814 (Q1, percentile 97%, CiteScore 12.5, SJR 0.69). 2) Kekibayeva A.K., Asrandina S.Sh., Atabayeva S.D., Baigaziyeva G.I., Nygymetova A.K. "Application of Stevia extract in the production of isotonic beverages" // Bulletin of Shakarim University, Technical Sciences Series. No. 1 (21) 2026. Status: In press, scheduled for release — March 2026 (Certificate/confirmation from the editorial board is available). 3) Asrandina S.Sh., Kekibayeva A.K., Atabayeva S.D., Kenzhebayeva S.S., Doktyrbay G. "Study of physicochemical and organoleptic indicators of functional beverages enriched with Stevia concentrates" // Bulletin of the Almaty Technological University, Food and Processing Industry Technology Series. Status: Under review. 4) Nygymetova A.K., Asrandina Sh.S., Baigaziyeva G.I., Atabayeva S.D., Kekibayeva A.A. "Calculation of a water-ethanol mixture for the preparation of an extract based on Stevia leaves" // IX International Scientific and Practical Conference "Scientific Foundations for Increasing the

	Efficiency of Agricultural Production". November 28, 2025. Kharkiv. – P. 272-275.
Patents	Method for producing a functional isotonic beverage. Registration No. 2026/0291.1 dated March 10, 2026 (currently under formal examination).