ABSTRACT

of the dissertation candidacy for the degree of Doctor of Philosophy (PhD)
in the specialty **6D060700 – "Biology"**

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«Study of cytoembryological and biochemical regularities of accumulation and analysis of natural rubber in tau saghyz population (Scorzonera tau-saghyz Lipsch. et G.G. Bosse) »

General characteristics of the research: The work is devoted to the study of the geographical distribution of tau-saghyz populations in the mountainous region of Karatau, the physicochemical analysis of natural rubber, the investigation of morphological, biochemical, and cytoembryological patterns (chromosome number) associated with its accumulation, the molecular phylogenetic identification of tau-saghyz, and the assessment of the effect of biohumus on plant growth and development.

Relevance of the research topic: Rubber is a polymeric material of strategic importance and is widely used in the manufacture of automobile tires, medical products, conveyor belts, sealants, shock absorbers, and other products intended for operation under dynamic loads. Rubbers are classified into natural and synthetic types, with more than 200 varieties produced industrially. In 2025, the synthetic rubber market reached USD 34.8 billion, accounting for approximately 60% of the more than 25 million tons of elastomers produced annually. However, the complete replacement of natural rubber with synthetic alternatives in several strategically important industries remains technologically impossible due to its unique physicochemical and mechanical properties. Natural rubber (*cis*-1,4-polyisoprene) is a polymer obtained from latex-producing plants and is characterized by high elasticity, strength, and resistance to mechanical stress. The annual global production volume is approximately 11–15 million tons. The principal commercial source of natural rubber is *Hevea brasiliensis*, which accounts for more than 90% of global production. However, the reliance on *Hevea brasiliensis*, a tropical tree highly vulnerable to phytopathogens, poses significant risks to the stability of natural rubber production and global supply chains. Given the limited raw material base, the search for alternative rubber-producing plants capable of synthesizing *cis*-1,4-polyisoprene has become particularly important. One promising species is *Scorzonera tau-saghyz*, a perennial semi-shrub of the Asteraceae family native to Central Asia. Its roots accumulate rubber comparable in quality to that obtained from *Hevea*. At present, tau-saghyz is considered a rare and endangered species with a narrow natural distribution range and is included in the Red Book of the Republic of Kazakhstan. However, the low seed germination rate, high mortality of young seedlings, and the use of insufficiently effective cultivation practices have become major factors hindering the large-scale plantation production of tau-saghyz roots. Furthermore, the discovery and industrial production of synthetic rubber in the mid-20th century led to the closure of all collective farms engaged in the cultivation of domestic rubber-producing plants, including tau-saghyz and kok-saghyz. In this regard, the search for scientific approaches to improve the seed germination of tau-saghyz and its subsequent development under plantation conditions is highly relevant, including the development of new agrotechnical cultivation methods capable of ensuring the sustainable production of natural rubber in the required volumes both in Kazakhstan and worldwide.

Aim: The study of the geographical distribution of tau-saghyz populations in the Karatau Mountains and their morphological and genetic characteristics. The development of

effective methods for the extraction of natural rubber from tau-saghyz roots and the evaluation of its physicochemical properties. Molecular phylogenetic identification and the determination of factors influencing rubber accumulation, including physiological and biochemical parameters as well as plant cultivation conditions.

Objectives:

1. To determine the geographical distribution of tau-saghyz populations in the Karatau mountain region, develop a method for the extraction of natural rubber from dried tau-saghyz roots, and conduct its physicochemical analysis.
2. To investigate the morphological, cytoembryological, and biochemical patterns of natural rubber accumulation in tau-saghyz roots.
3. To carry out the molecular phylogenetic identification of wild tau-saghyz populations growing in the Karatau Mountains.
4. To study the effects of dry vermicompost and vermicompost tea on the growth and development of tau-saghyz under laboratory conditions.

Objects of the study: Samples from populations of *Scorzonera tau-saghyz* (tau-saghyz) growing in the Karatau Mountains.

Research methods: The study employed field, morphological, cytological, molecular genetics, biochemical, and physicochemical research methods. The extraction and analysis of natural rubber were carried out using extraction and chromatographic techniques.

Scientific novelty of the research: For the first time, based on the study of geographical distribution, it was established that *Scorzonera tau-saghyz* is characterized by pronounced ecologically determined intraspecific variability. A high level of intraspecific variation in root rubber content was revealed, indicating the presence of promising high-yielding forms for breeding purposes. For the first time, an elemental analysis of rubber extracted from tau-saghyz roots was conducted, including the determination of macro elements (Ca, K, Mg) and microelements (Zn, Fe). Using size-exclusion chromatography, thermogravimetric analysis, Fourier-transform infrared spectroscopy, differential thermogravimetric analysis, and nuclear magnetic resonance, a molecular weight of 1223.9 Mw (kg/mol) and a *cis*-1,4-polyisoprene structure were established. The potential of aqueous extraction of rubber from dry roots was demonstrated as an environmentally friendly and cost-effective method compared to hexane extraction. It was shown that the level of rubber accumulation in roots during the flowering phase is independent of photosynthetic pigment content. It was also established that tau-saghyz has a diploid chromosome number (2n = 14). The detected SNP polymorphisms in the ITS1 region of cDNA, validated using the dCAPS method, indicate the presence of genetic heterogeneity within *Scorzonera tau-saghyz* populations. The observed initial differentiation may be of practical interest for breeding programs aimed at selecting genotypes with improved agronomic traits, including seed germination and stress tolerance. For the first time, the effectiveness of vermicompost and vermicompost tea in enhancing seed germination, plant growth, and biomass accumulation has been demonstrated.

Scientific and practical significance of the research: The obtained results provide a basis for improving technologies for the production of natural rubber and are of significant importance for the development of alternative sources capable of partially replacing *Hevea brasiliensis*.

Main provisions put forward for defense:

1. Populations of tau-saghyz growing in the Karatau mountain region of the Republic of Kazakhstan are characterized by pronounced ecologically determined intraspecific variability, manifested in the variation of the root system, caudex, and vegetative and

generative organs (seeds). The latex and rubber of tau-saghyz contain a statistically confirmed higher content, compared to kok-saghyz, of the following macroelements (Ca, K, Mg) and microelements (Zn, Fe). Using SEC, NMR, FT-IR, TGA, and DTGA methods, a high molecular weight of rubber (Mw = 1223.9 kg/mol) and a *cis*-1,4-polyisoprene structure were established.

2. It was established that the diploid chromosome number of tau-saghyz is 2n = 14. The level of rubber accumulation in roots during the flowering phase (May) does not depend on the content of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids). Rubber biosynthesis in roots begins at the onset of the summer dormancy period (July–August).

3. SNP polymorphisms in the ITS1 region of cDNA were identified using dCAPS molecular markers and reflect an initial level of genetic differentiation among *Scorzonera tau-saghyz* populations.

4. The effectiveness of vermicompost and vermicompost tea is expressed in an increase in seed germination by 68.3 ± 4.68%. In the control group, the average mass of the aboveground part of *S. tau-saghyz* was 83.3 ± 6.3 mg, whereas in the experimental group it was three times higher, averaging 252 ± 18.1 mg. In another comparison, the average shoot mass in the control group was 220 mg, while in the experimental group it was 476 mg, which is 2.19 times higher.

Author's personal contribution: The dissertation is a completed scientific work. The author participated in all stages, including the analysis of literature data, formulation of the research aims and objectives, selection of methods, conducting field and experimental studies, processing and discussion of results, preparation of conclusions and recommendations, and writing of the manuscript.

Connection of the research with state programs: The research presented in the dissertation was carried out within the framework of a grant-funded research project: AP05134291, "Genome sequencing of *Scorzonera tau-saghyz Lipsch*. et G.G. Bosse and genome-wide association mapping for rubber content traits." The scientific supervisor was Doctor of Biological Sciences, Professor K.K. Boguspaev.

Approbation of the research work:

The main results and findings of the dissertation research were presented at international scientific conferences and published in the following proceedings and collections:

1. International Conference of Students and Young Scientists "Farabi Әlemi" (2018, Almaty, Kazakhstan);
2. International Scientific and Practical Conference "Current Issues of Ecological Genetics and Experimental Biology" (January 25, 2018, Almaty, Kazakhstan);
3. International Conference of Students and Young Scientists "Farabi Әlemi" (April 6–9, 2020, Almaty, Kazakhstan);
4. International Scientific and Practical Conference **"**Modern Problems of Biotechnology: from Laboratory Research to Production" (June 4–5, 2021, Almaty, Kazakhstan);
5. Proceedings of the Round Table "Interdisciplinary Study of Genetic Processes: Present and Future" (2023, Almaty, Kazakhstan);
6. International Scientific and Practical Conference "Environmental Biotechnology and Innovations in Bioenergy" (February 12–13, 2021, Almaty, Kazakhstan);
7. International Conference of Students and Young Scientists "Farabi Әlemi" (April 10–11, 2019, Almaty, Kazakhstan);
8. International Symposium "Astana Biotech 2018" (2018, Astana, Kazakhstan).

Publications:
The main content of the dissertation has been published in 18 scientific works, including 5 articles in international journals indexed in the *Scopus* database with high impact factors, and 3 national intellectual property patents. Two articles were published in scientific journals included in the list of the Committee for Quality Assurance in Education and Science, along with 8 abstracts presented at international conferences and symposia. The author independently conducted the literature review on the studied problems, defined the research aims and objectives, carried out experimental studies, performed statistical processing and analysis of the results, and wrote the dissertation.

Main conclusions:

1 The geographical distribution of *Scorzonera tau-saghyz* populations in the Karatau Mountains, habitat ecology, and soil agrochemical properties were investigated. Ecologically driven morphological variation of the species was revealed. Mechanical water-based and hexane extraction methods for natural rubber from dried roots were developed. FTIR, SEC, NMR, TGA, and DTGA analyses confirmed the *cis*-1,4-polyisoprene structure, high molecular weight (Mw = 1223.9 kg/mol), and the presence of the major macroelements (Ca, K, Mg) and microelements (Zn, Fe) in the rubber.

2 The morphological, cytogenetic, and biochemical characteristics of natural rubber accumulation in the roots of *Scorzonera tau-saghyz* were investigated. The species was confirmed to possess a diploid chromosome number (2n = 14). Rubber content ranged from 8.3% to 38.7% of root dry weight and depended on plant morphology and environmental conditions. No significant correlation was found between photosynthetic pigment content and rubber accumulation.

3 Molecular phylogenetic identification of *Scorzonera tau-saghyz* populations was performed using ITS1 nucleotide sequences. SNP polymorphisms were validated by dCAPS markers, revealing initial genetic differentiation among populations and relatively higher genetic variability in the eastern Karatau population.

4 The positive effects of vermicompost and vermicompost tea on seed germination and seedling growth of *Scorzonera tau-saghyz* were experimentally demonstrated. Their application enhanced seed germination, root and shoot development, and biomass accumulation, providing a scientific basis for improving cultivation technologies of this species.

Structure of the dissertation: The dissertation consists of 122 pages of computer-typed text, including symbols and abbreviations, and comprises an introduction, literature review, materials and methods section, results and discussion, conclusion, and references. The work includes 14 tables, 43 figures, and 1 appendices.