

Research Article

<https://doi.org/10.31489/2026FEB2/185-191>

UDC 581.142

Received: February 05, 2026 | Accepted: April 01, 2026 | Published online: June 30, 2026

E.A. Gavrilkova*, S.S. Tyrzhanova, S.U. Tleukenova, R.T. Mussina

Karaganda National Research University named after Academician E.A. Buketov, Karaganda, Kazakhstan

*Corresponding author: elena_2809@mail.ru

Cryopreservation of *Filipendula ulmaria* Seed Material

Cryopreservation of plant seeds is of great practical importance for biodiversity conservation and practical application. The aim of this study was to evaluate the factors determining the success of cryopreservation of *Filipendula ulmaria* seeds, including the freezing method, thawing conditions, storage containers, and light sensitivity. Seed material was collected in the wild in the Karkaraly Mountains. Cryopreservation was carried out using cryotubes and aluminum foil bags while seeds stored using traditional methods in a refrigerator served as controls. The results showed no significant differences in germination between light and dark conditions, which does not dictate the method of germination in open ground or laboratory conditions. Comparison of thawing regimes revealed the advantage of slow thawing at room temperature (20–22 °C), during which germination reached 75 %, which was significantly higher than the control values. Analysis of freezing regimes allowed us to establish the highest germination rates with rapid freezing in foil bags. The proposed approach can be used for long-term cryopreservation of the *Filipendula ulmaria* gene pool.

Keywords: *Filipendula ulmaria*, seed material, cryopreservation, seed germination, germination energy, freezing-thawing regimen.

Introduction

The conservation of biological diversity and the establishment of seed banks for economically valuable plants are of significant scientific and practical importance [1]. This need arises from land degradation caused by human activity, as well as global climate change [2]. Among various storage methods, cryopreservation stands out as one of the most effective [3, 4]. Seed preservation creates potential for plant breeding [5, 6]. Storing plant material at -196 °C temperature stops all metabolic and physiological processes [5]. Storage at extremely low temperatures allows genetic material to be preserved indefinitely, with minimal financial costs and maintenance, without requiring large areas of space. Literature data [3–8] demonstrate the effectiveness of cryopreservation for medicinal plant seeds.

An interesting subject for research is *Filipendula ulmaria* (L.) Maxim (family Rosaceae), whose herb is used in folk medicine as an anti-inflammatory, diuretic, antirheumatic, antibacterial, antioxidant, and analgesic agent, as well as for digestive problems, respiratory diseases, and skin conditions [9–11].

To introduce medicinal plant seed material into a cryobank, studies were conducted to optimize the cryopreservation conditions for *Filipendula ulmaria* seeds.

Experimental

The subject of the laboratory studies was *F. ulmaria* seeds. The plant is frost-tolerant, drought-tolerant, and a facultative heliophyte, preferring xeromesophilic habitat conditions and moderately moist loamy or humus-rich sandy loam soils (Fig. 1). Under the conditions of Central Kazakhstan, the species is successfully introduced and can be cultivated on an industrial scale, which creates a need for the establishment of a seed bank, including storage at subcritical low temperatures.



Figure 1. Flowering shoot of *Filipendula ulmaria*

The research material was collected in the Karkaraly Mountains (Karkaraly District, Karaganda Region) during the third decade of August 2024.

Laboratory studies on optimizing cryopreservation conditions for *F. ulmaria* seed material were conducted at the Research Park for Biotechnology and Eco-Monitoring of the Faculty of Biology and Geography (Karaganda National Research University named after academician E.A. Buketov) in 2023–2025.

Prior to freezing, the initial moisture content of the seeds was determined using an Ohaus (China) moisture meter. *F. ulmaria* seeds were stored in an SDS-20 CryoMach (Russia) Dewar flask with liquid nitrogen ($-196\text{ }^{\circ}\text{C}$) for one month [12]. Aluminum foil bags and cryotubes (Deltalab, China) were used as freezing containers [13]. *F. ulmaria* seed material was not specifically selected prior to the start of the experiments; damaged, immature, and empty seeds were removed.

Seeds were thawed after cryopreservation using the following methods:

- 1) slow de-frosting at room temperature ($20\text{--}22\text{ }^{\circ}\text{C}$);
- 2) fast de-frosting in a water bath ($40\text{--}45\text{ }^{\circ}\text{C}$).

Stepwise freezing and rapid freezing (by immersing the seeds in a container in liquid nitrogen) were also investigated [14].

To assess the effectiveness of cryopreservation of *F. ulmaria*, seed viability was determined based on laboratory germination and germination energy [15] in Petri dishes. Before sowing the seeds, the Petri dishes are treated with 90 % alcohol and placed in laminar flow cabinets for 20 minutes for additional disinfection. After this time, filtered discs moistened with distilled water are placed in the Petri dishes. Before sowing in Petri dishes, the seeds were treated with a 0.5 % potassium permanganate solution for 5 minutes, then rinsed with distilled water [16].

The Petri dishes were placed in a climate chamber for the entire duration of the study, at a temperature of $+24\text{ }^{\circ}\text{C}$ and under constant illumination. Seeds were watered with distilled water as needed, if the Petri dish substrate dried out. The lids of the Petri dishes had to be opened for 5–10 seconds daily to allow the gas composition inside the dishes to change. During germination observation, the emergence of seedlings was recorded daily; germination rate was assessed on day 15, and germination energy on day 7. Seeds stored using the traditional method (refrigerator, $0\text{--}2\text{ }^{\circ}\text{C}$) served as the control.

All experiments were conducted in four independent replicates ($n=4$) by 100 seeds. Data are presented as mean \pm standard deviation (SD). Differences between treatments were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test at a significance level of $p < 0.05$. All statistical analyses were performed using R software (R Foundation for Statistical Computing, Vienna, Austria).

Results and Discussion

In the initial stages of the study, the germination of seeds stored without cryopreservation was analyzed. Seed germination was conducted in light and in the dark. It was found that for *F. ulmaria* seed material, no significant difference was detected between the light and dark germination treatments. Seed germination was $71.0 \pm 5.4\%$ in light and $70.0 \pm 3.54\%$ in darkness (Tab. 1).

Seed germination of *Filipendula ulmaria* under various lighting conditions

Germination rates, %	Germination conditions	
	In light	In the dark
Germination energy	65.6±1.25	68.4±2.25
Seed germination	71.0±5.4	70.0±3.54

The data obtained indicate that there are no requirements for deep sowing for this species.

When seeds are stored in liquid nitrogen, the container [5] in which freezing takes place affects the germination of the plant material. The seed material was packaged in foil envelopes and plastic cryotubes. The results showed that the seeds retain their germination capacity after storage in liquid nitrogen vapors with complete physiological ripening (Fig. 2).

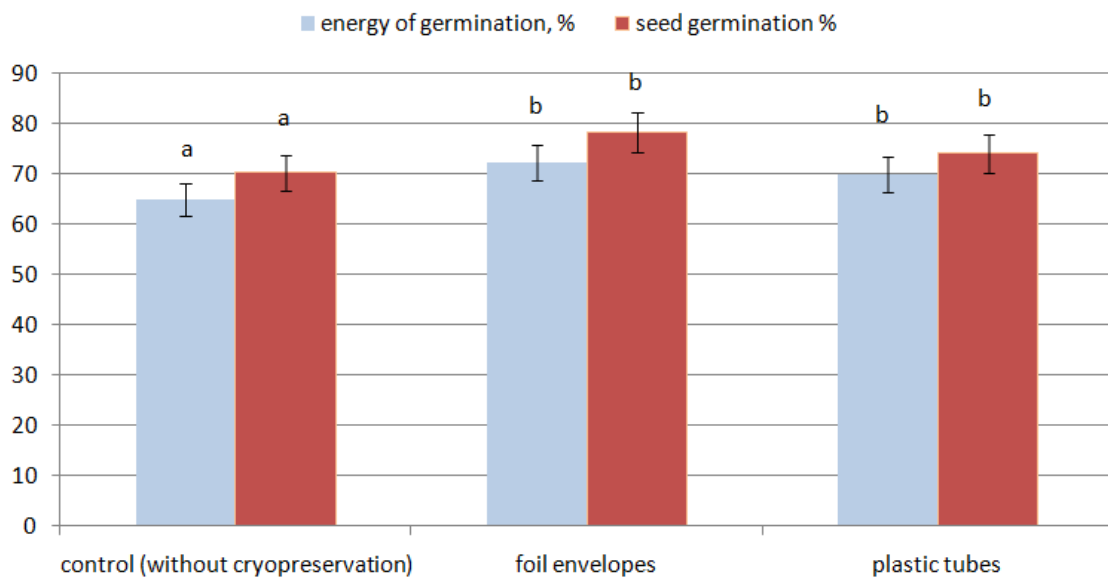


Figure 2. Germination of *Filipendula ulmaria* seeds in various containers. Different letters indicate significant differences between treatment groups at $P \leq 0.05$

The highest germination energy and seed germination were observed in *F. ulmaria* seeds stored at liquid nitrogen temperature in foil envelopes — 78.25 ± 8.41 %. The germination of seed material frozen in cryotubes were 75.0 ± 7.45 %, which is significantly higher than the control and does not differ significantly from the results obtained in foil containers. Thus, the germination rate of seed material in the cryotube experiments was 5.0 % higher than the control, and in the foil envelope variant, it was 8.25 % higher.

Thus, when storing *F. ulmaria* seed material in liquid nitrogen, both foil envelopes and cryotubes are recommended containers.

The next factor influencing the success of seed preservation during cryopreservation is the thawing conditions. An analysis of the literature [5–11] showed that thawing conditions may vary for different plant species, depending on genetic characteristics, seed coat thickness, physiology, and the accumulation of reserve nutrients.

During laboratory studies, two thawing regimes were initially used: slow thawing at room temperature (+20–22°C) and fast thawing in a water bath (+40°C).

Slow thawing at a temperature of +22–24 °C following cryogenic storage proved to be the significantly better option for *F. ulmaria* seeds (75.0 ± 7.45 %). This method exceeded the control values by 5.0 %. With rapid thawing, the germination rate of the seeds of the studied species was 67.5 ± 5.53 %, which was 2.5 % lower than the control values; however, no statistically significant differences from the control were detected (Fig. 3).

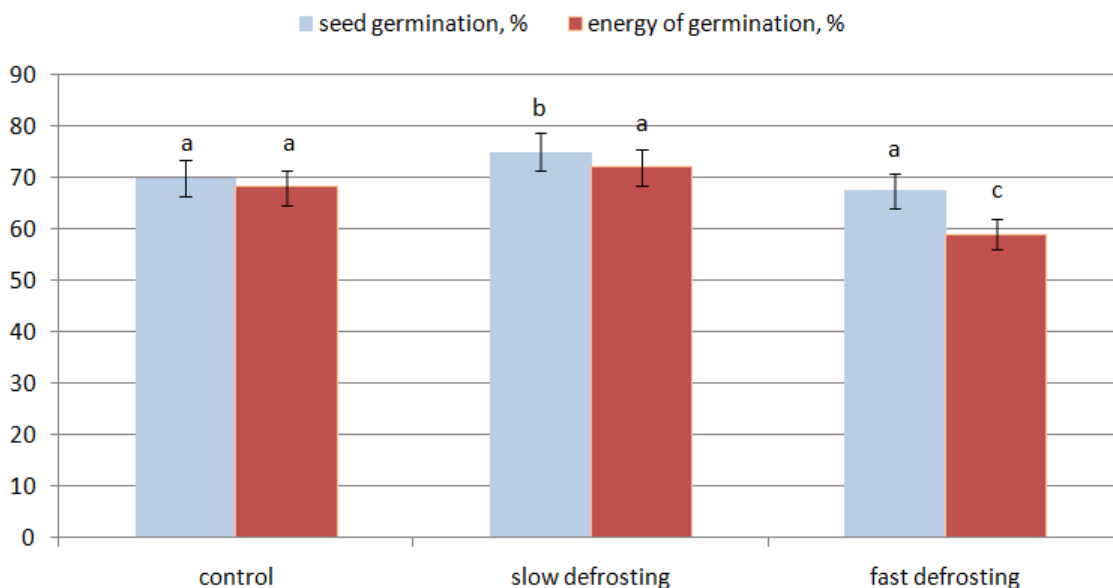


Figure 3. Germination rates of *Filipendula ulmaria* seeds depending on the thawing regime. Different letters indicate differences between experimental variants at $P \leq 0.05$

Thus, to achieve the best germination rates and germination energy of *F. ulmaria* seeds, slow thawing at room temperature (+22–24 °C) is recommended.

In the next stage, experiments were conducted to compare stepwise and rapid freezing. Rapid freezing was performed by quickly immersing plant samples in cryotubes and foil envelopes in liquid nitrogen for three days. Two-stage freezing was also performed in foil envelopes and cryotubes.

First, the test samples were placed in a refrigerator at +4 °C for 60 minutes, and then transferred to a freezer at -20 °C for one hour. Next, the test seeds in their containers were immersed in the vapor of liquid nitrogen contained in a Dewar flask for three days. After the seed material of the test species had been in liquid nitrogen, a slow thawing mode was used.

Based on the results of the experimental studies, it was determined that when seed material is rapidly placed in liquid nitrogen vapor, foil envelopes are the best container for achieving the highest germination rate (Fig. 4). Seed germination in this experimental treatment was 78.25 ± 8.41 %. For seeds frozen in plastic containers, germination energy did not differ significantly from the control, while germination was significantly higher than the control but lower than the values obtained for the foil container treatment.

In the two-stage freezing of *F. ulmaria* seeds, the germination energy values for plastic containers were significantly lower than the control, whereas for foil containers, they did not differ significantly from the control. However, seed germination for both container variants was significantly higher than the control.

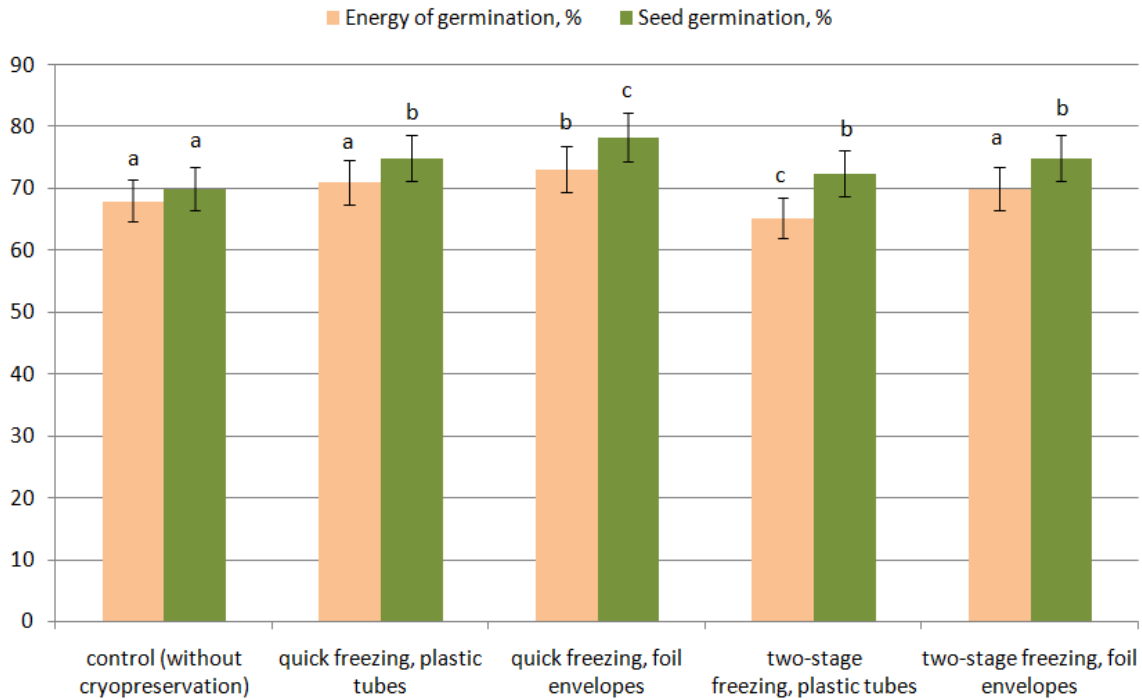


Figure 4. Germination of *Filipendula ulmaria* seeds depending on the freezing regime. Different letters indicate significant differences between experimental variants at $P \leq 0.05$

A comparative analysis of the two freezing methods used revealed that the highest germination rates were observed for the variant involving freezing in foil containers and the use of the rapid method. This result can be explained by the fact that foil provides faster and more uniform cooling, and the rapid method prevents the formation of ice crystals in the tissues.

Conclusion

Thus, for long-term cryopreservation, it is recommended to freeze *F. ulmaria* seeds in foil envelopes by rapid immersion in a Dewar flask and to use a slow thawing regimen. The results demonstrated the effectiveness of this storage method, as *F. ulmaria* seeds successfully survived storage at supercritical low temperatures, not only maintaining but also increasing germination rates and germination energy.

The data obtained can be used to develop a cryopreservation protocol and introduce *F. ulmaria* into a seed cryobank.

Funding

This study was conducted within the framework of targeted funding from the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (BR28712367 “Comprehensive study of non-traditional wild medicinal plants, mobilization of their genetic resources in botanical gardens of Western, Eastern, and Central Kazakhstan, creation of a bioinformatics database for the development of para-pharmaceutical products,” 2025–2027).

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Gavrilkova E.A.** — conceptualization, laboratory experiments, investigation; **Tyrzhanova S.S.** — seed collection, draft writing; **Tleukenova S.S.** — data analysis; **Mussina R.T.** — data curation, data collection, draft writing.

Conflict of Interest

The authors declare no conflict of interest.

References

- 1 Nakum, P., Singh, B., & Solanki, H. (2025). The significance of seed banks: A review of techniques and technologies. *World Journal of Advanced Research and Reviews*, 25(02), 2401–2407. <https://doi.org/10.30574/wjarr.2025.25.2.0573>
- 2 Wambugu, P.W., Nyamongo, D.O., & Kirwa, E.C. (2023). Role of Seed Banks in Supporting Ecosystem and Biodiversity Conservation and Restoration. *Diversity*, 15(8), 896. <https://doi.org/10.3390/d15080896>
- 3 Zeng, L., Sun, Z., Fu, L., Gu, Y., Li, R., He, M., & Wei, J. (2024). Cryopreservation of Medicinal Plant Seeds: Strategies for Genetic Diversity Conservation and Sustainability. *Plants*, 13(18), 2577. <https://doi.org/10.3390/plants13182577>
- 4 Pavlov, A.V., Porokhovinova, E.A., Pavlov, A.V., Kiseleva, I.V., & Brutch, N.B. (2026). The Influence of Cryopreservation and Low-Temperature Seed Storage on the Morphological and Agronomical Characteristics of Fiber Flax. *Plants*, 15(4), 602. <https://doi.org/10.3390/plants15040602>
- 5 Ishmuratova, M., Tleukenova, S., Ramazanov, A., Gavrilkova, E., & Ageev, D. (2021). Study of cryopreservation conditions for seed materials of *Chartolepis intermedia* Boiss. *Bio Web of Conferences — Northern Asia Plant Diversity*, 38, 00046. <https://doi.org/10.1051/bioconf/20213800046>
- 6 Ren, R., Zhe, J., Guo, J., & Yang, X. (2023). Cryopreservation of herbaceous Asteraceae seeds: effects of seed reserves on seed germination and seedling regrowth. *Cryobiology*, 112, 104562. <https://doi.org/10.1016/j.cryobiol.2023.104562>
- 7 Kholina, A.B., & Voronkova, N.M. (2012). Seed cryopreservation of some medicinal legumes. *Journal of Botany*, 2012(1), 186891. <https://doi.org/10.1155/2012/186891>
- 8 Ghaffarzadeh-Namazi, L., Babaeian, N., Ghamari-zare, A., & Nematzadeh, G.H. (2015). Cryopreservation of the seeds of the medicinal plant *Satureja bachtiarica* Bunge. *Int J Biosci.*, 6(2), 24–29. <https://dx.doi.org/10.12692/ijb/6.2.24-29>
- 9 Farzaneh, A., Hadjiakhoondi, A., Khanavi, M., Manayi, A., Bahram Soltani, R., & Kalkhorani, M. (2021). *Filipendula ulmaria* (L.) Maxim. (meadowsweet): a review of traditional uses, phytochemistry and pharmacology. *Research J Pharmacognosy*, 9(3), 85–106. <https://doi.org/10.22127/rjp.2021.302028.1781>
- 10 Andonova, T., Muhovski, Y., Apostolova, E., Naimov, S., Mladenova, S., Slavov, I., Dincheva, I., Georgiev, V., Pavlov, A., & Dimitrova-Dyulgerova, I. (2024). DNA-Protective, Antioxidant, and Anti-Carcinogenic Potential of Meadowsweet (*Filipendula ulmaria*) Dry Tincture. *Antioxidants*, 13(10), 1200. <https://doi.org/10.3390/antiox13101200>
- 11 Samardzic, S., Arsenijevic, J., Bozic, D., Milenkovic, M., Tesevic, V., & Maksimovic, Z. (2017). Antioxidant, anti-inflammatory, and gastroprotective activity of *Filipendula ulmaria* (L.) Maxim. and *Filipendula vulgaris* Moench. *Journal of Ethnopharm.*, 213, 132–137. <https://doi.org/10.1026/j.jep.2017.11.013>
- 12 Sharma, M., Kumar, P., & Dwivedi, P. (2023). Artificial seed production and cryopreservation technology for conservation of plant germplasm with special reference to medicinal plants. *Medicinal Plants: Biodiversity, Biotechnology and Conservation*, 33, 581–593. Springer.
- 13 Reed B.M. (2008). *Plant cryopreservation: a practical guide*. Springer Science and Business Media, USA.
- 14 Shri, K.K., Pavithra A., Pazhamalai, V., Meenambiga, S.S., & Romauld, S.I. (2025). Plant genetic resources conservation by cryopreservation. In *Biotechnology in Plant Conservation*. Springer.
- 15 Hegde, R.B., Kudva, V., & Nayak, S. (2025). Advanced techniques for seed quality assessment and germination monitoring. *Discov Appl Sci.*, 690 (2025). <https://doi.org/10.1007/s42452-025-07284-8>
- 16 Prasad, S.R. (2023). Testing seed for quality. In *the book Seed Science and Technology, 2023*, 299–334. https://doi.org/10.1007/978-981-19-5888-5_13

Е.А. Гаврилькова, С.С. Тыржанова, С.У. Тлеукенова, Р.Т. Мусина

***Filipendula ulmaria* тұқымдық материалын криоконсервациялау**

Өсімдік тұқымдарын криоконсервациялау биоәртүрлілікті сақтауда практикалық маңызға ие. Зерттеудің мақсаты *Filipendula ulmaria* тұқымдарын криоконсервациялаудың факторларына, соның ішінде мұздату әдісіне, жібіту режиміне және ыдыстар түрлеріне, сонымен қатар жарыққа байланысты баға беру. Тұқымдық материалы Қарқаралы тауларында табиғи жағдайда жиналды. Тұқымдар криодыстарда және алюминий фольга пакеттерінде криоконсервацияланды, ал бақылау ретінде тоңазытқыш камерада дәстүрлі тәсілмен сақталған тұқымдар алынды. Зерттеу нәтижелері жарықта және қараңғыда өсу кезінде айтарлықтай дәлелді айырмашылықтардың болмауын көрсетті, бұл ашық топырақ пен зертханалық жағдайларда өсіруді шектемейді. Жібіту режимдерін салыстыру бөлме температурасында (20–22 °C) баяу жібітудің артықшылығын көрсетті, онда өнгіштік 75 % құрады, бұл бақылау мәндерінен айтарлықтай жоғары болып шықты. Мұздату режимдерін талдауда фольга пакеттерінде жылдам мұздату кезінде ең жоғары көрсеткіштерді анықтауға мүмкіндік берді. Ұсынылған тәсіл *Filipendula ulmaria* генофондын ұзақ мерзімді криосақтау үшін пайдаланылуы мүмкін.

Кілт сөздер: *Filipendula ulmaria*, тұқымдық материал, криоконсервациялау, тұқымның өнгіштігі, өну энергиясы, мұздату-жібіту режимі.

Е.А. Гаврилькова, С.С. Тыржанова, С.У. Тлеукунова, Р.Т. Мусина

Криоконсервация семенного материала *Filipendula ulmaria*

Криоконсервация семян растений имеет важное практическое значение для сохранения биоразнообразия и их использования. Целью настоящего исследования являлась оценка факторов, определяющих успешность криоконсервации семян *Filipendula ulmaria*, включая способ замораживания, режим оттаивания, тару и отношение к свету. Семенной материал был собран в природных условиях в горах Каркаралы. Семена криоконсервировали в криопробирках и в пакетах из алюминиевой фольги, контролем служили семена, хранившиеся традиционным способом в холодильной камере. Результаты показали отсутствие достоверных отличий между проращиванием на свету и в темноте, что не регламентирует способ проращивания в открытом грунте и лабораторных условиях. Сравнение режимов оттаивания выявило преимущество медленного размораживания при комнатной температуре (20–22°C), при котором всхожесть составила 75 %, что оказалось достоверно выше контрольных показателей. Анализ режимов замораживания позволил установить максимальные показатели при быстром замораживании в пакетах из фольги. Предлагаемый подход может быть использован для долгосрочного криохранения генофонда *Filipendula ulmaria*.

Ключевые слова: *Filipendula ulmaria*, семенной материал, криоконсервация, всхожесть семян, энергия прорастания, режим замораживания — оттаивания.

Information about the authors

Gavrilkova Elena Anatoljevna — Master of Biology, Senior Lecturer, Karaganda National Research University named after Academician E.A. Buketov, Karaganda, Kazakhstan; e-mail: elena_2809@mail.ru; ORCID: <https://orcid.org/0000-0001-7077-9636>

Tyrzhanova Sayagul Serikovna — PhD, Senior Lecturer, Department of Zoology, Karaganda National Research University named after Academician E.A. Buketov, Karaganda, Kazakhstan; e-mail: tssaya@mail.ru; ORCID: 0000-0002-6544-1726

Tleukenova Saltanat Ushkempirovna — Candidate of Biological Sciences, Head of the Botany Department, Karaganda National Research University named after Academician E.A. Buketov, Karaganda, Kazakhstan; e-mail: saltauniq@mail.ru; ORCID: 0000-0002-4714-0110

Mussina Rosa Tletayevna — Master of Ecology, Lecturer, Department of Botany, Karaganda National Research University named after Academician E.A. Buketov, Karaganda, Kazakhstan; e-mail: rosanna_1983@mail.ru; ORCID: <https://orcid.org/0009-0005-9207-8984>