

ABSTRACT

dissertation for the degree of Doctor of Philosophy (PhD)
in the educational program 8D05101 – Biology

Madiyeva Aida Narimanovna

Cryopreservation of seed material of Sudan grass varieties (*Sorghum sudanense* L.) and development of methods for short-term and long-term storage

General characteristics of the work. The dissertation work is devoted to the study of cryopreservation conditions of seed material of varieties of Sudan grass and the development of short- and long-term storage methods.

The relevance of the topic. The creation of a stable and highly nutritious food supply is one of the main factors in ensuring the productivity of farm animals.

Increasing the productivity of livestock products will make it possible to more fully supply the domestic market of Central Kazakhstan with food of animal origin, and increase its export potential and competitiveness, as well as create additional jobs [1].

This will increase the range of fodder crops with new crops, which will help strengthen the feed base for livestock production in the region, and reduce the cost of livestock products [1].

It is worth noting that most of the territory of Kazakhstan is located in arid conditions, characterized by high temperatures in summer and a lack of precipitation.

In recent years, the problem of climate change has become more pronounced and has become a significant factor affecting various spheres of human activity. Its consequences are particularly acute in agriculture, where the level of crop production directly depends on natural and climatic conditions [2, 3]. The development of the agricultural sector is largely associated with an increase in the efficiency of animal husbandry, which, in turn, is impossible without sufficient provision of feed resources. In arid regions, the formation of a stable food supply is difficult, as a result of which there is a shortage of feed, most noticeable in the second half of the summer period [4].

Significant changes in climatic conditions have led to a shift in the boundaries of agro-climatic zones, the formation of which is associated with the duration of the active vegetation period, determined by the sum of temperatures above 10 ° C. The observed increase in average temperatures, a decrease in precipitation, as well as an increase in the frequency and duration of droughts negatively affect crop productivity and worsen the quality of products [2, 5].

With the intensification of animal husbandry, it becomes necessary to simultaneously increase the production of affordable feed and ensure their high quality on an ongoing basis [2].

It is possible to increase production efficiency in a comprehensive manner: along with improving the farming system, it is important to select crops and varieties correctly, as well as take into account the peculiarities of agro-climatic zones when they are located on the territory of the republic. This approach helps to reduce the adverse effects of weather factors [2, 6].

When there is a shortage of moisture, fodder crops that can produce stable and high yields, as well as containing large amounts of digestible protein, are of particular importance. Among them, the Sudanese herb stands out [4].

Sudan grass is distinguished primarily by its drought resistance, high productivity, high quality of green mass, high sugar content and good digestibility by farm animals.

The success of introducing this crop into production practice is largely determined by the degree of development of the seed production system and the level of seed supply. The formation of own seed resources is a key factor ensuring its widespread use in feed production [2].

At the present stage of scientific and technological progress in the field of biotechnology, improved methods of low-temperature preservation of plant seed material are being introduced. This opens up opportunities for the formation of long-term genetic reserves and the rapid production of the required amount of planting material for the required crops. Cryopreservation is the storage of plant seeds at an ultra-low temperature (-196 ° C) for a long period of time while maintaining the viability of the seeds.

In Kazakhstan, the use of low-temperature storage technologies for seed remains underdeveloped. Scientific research concerns the cryopreservation of seeds of various plant groups, as well as individual biological objects, including meristems, pollen, buds and cuttings.

Previously, cryopreservation was practically not used for forage crops, and there are a limited number of literary sources. For example, there is data on the collection of crop samples at the Federal State Budgetary Institution "Federal Research Center All-Russian Institute of Plant Genetic Resources named after N.I.Vavilov" (VIR), where the collection of legumes and cereals is kept [7].

Cryopreservation of Sudan grass seeds has not been performed before.

The relevance of the study is the introduction of varieties of Sudan grass: Nika, Alina, Tugai, Novosibirskaya 84 in the territory of the Karaganda region as one of the most suitable drought-resistant crops for the arid region and the creation of a gene bank of seeds by cryopreservation using (selection) the optimal cryoprotector for each variety.

Thus, the implementation of scientific research on the development of the basics of seed conservation using cryopreservation methods is an important and relevant area aimed at ensuring the sustainable development of the agricultural sector in the region.

The purpose of the thesis is to study the effect of ultra-low temperatures on the seed material of varieties of Sudan grass Tugai, Nika, Novosibirskaya 84, Alina

(*Sorghum*drummondii* (Nees ex Steud.) Millsp. & Chase) and the development of short- and long-term storage methods.

Research objectives:

1. To select optimal conditions for thawing seeds in liquid nitrogen and optimal cryoprotectors for cryopreservation of seeds of 4 varieties of Sudan grass, as well as to determine their thawing modes after cryopreservation;

2. To determine the effectiveness of the effects of physical methods of pre-sowing treatment on seeds of the studied varieties using laser radiation, magnetic irradiation, and bubbling;

3. To establish the effects of short-term and long-term storage of seeds at ultra-low temperatures on the viability of seeds of the studied varieties;

4. To introduce plants of Sudan grass varieties Alina, Nika, Novosibirskaya 84, Tugai in the territory of Karaganda, to study their stages of ontogenesis, the rhythm of growth and development of Sudan grass, to assess the feed value of varieties of Sudanese grass in the Karaganda region;

5. To identify the features of the anatomical structure of stems and roots of 4 varieties of Sudanese grass before and after cryopreservation.

Objects of research: seeds of Sudan grass varieties "Nika", "Novosibirskaya 84", "Tugai", "Alina". The seeds were provided by LLP "Scientific and Production Center of Grain economy named after Baraev", Shortandy village, Akmola region. The seeds of the "Alina" variety were provided by the Zhezkazgan Botanical Garden of the Ulytau region by the branch of the RSE on PHB "Institute of Botany and Phytointroduction" of the KKHZHM MENR RK.

Research methods: assessment of seed germination and germination biology, cryopreservation of Sudan grass seeds by direct immersion in liquid nitrogen, thawing of seeds using two methods of thawing (slow and fast defrosting), assessment of the influence of physical methods on the germination of Sudanese grass seeds, such as laser radiation, magnetic field, bubbling, anatomical study of the stem structure and plant root, determination of the forage value of Sudan grass. Statistical processing and graphical visualization of the obtained data was carried out using the R-studio environment and Microsoft Excel software.

Scientific novelty of the research:

1. For the first time, work was carried out to study the viability of seed material of 4 varieties of Sudanese grass, morphometric and anatomical parameters of seedlings before and after cryopreservation were studied;

2. For the first time, the effect of cryoprotectors on the germination and germination energy of Sudanese grass seeds was revealed, and the most suitable cryoprotector for each of the varieties was determined.

3. For the first time, the influence of physical factors on the germination of Sudan grass seeds, such as magnetic field, laser irradiation, and bubbling, has been studied.

4. For the first time, plants of Sudan grass varieties Alina, Nika, Novosibirskaya 84, Tugai were introduced in the territory of Karaganda, the phenological phases of

the development of Sudan grass in new conditions were studied, and the forage value of Sudanese grass varieties in the Karaganda region was assessed.

Theoretical and practical significance. A study of the conditions of cryopreservation of seeds of varieties of Sudan grass has shown that plastic cryoprobes are the optimal container for cryofreezing.

It was determined that the cryopreservation of Sudan grass seeds should use the method of slow defrosting at room temperature.

It has been proven that seeds of Alina and Nika varieties can be stored at ultra-low temperatures without loss of viability for up to 9 months, while short-term storage of no more than 3 months is recommended for Tugai and Novosibirskaya 84 varieties due to a decrease in germination with longer cryopreservation.

It is recommended to use DMSO, PVS2, ethylene glycol, propylene glycol as cryoprotectors. It is not recommended to use glycerin as a cryoprotector.

It has been revealed that pre-sowing treatment of seeds by physical methods of treatment has a positive effect on germination and germination energy of seeds of Sudanese grass.

It was determined that after exposure to ultra-low temperatures in the anatomical structure of cells between control plant variants and after cryopreservation, there are significantly significant differences in the size of root xylem cells, stem xylem cells, stem parenchyma cells, stem epidermis cells.

Main results:

1. It was determined that the freezing of seeds of Sudan grass varieties Alina and Nika is effective both in foil envelopes and in plastic tubes, thawing seeds is effective both at room temperature and in a water bath. For Tugai and Novosibirskaya 84 varieties, the optimal defrosting method is slow defrosting at room temperature, since under rapid defrosting conditions, there is a decrease in germination and seed germination energy. The most optimal cryoprotectors for varieties of Sudan grass are DMSO, PVS2, ethylene glycol, propylene glycol. We do not recommend using glycerin, sucrose and glucose for the cryopreservation of Sudan grass seeds.

2. The magnetic field, laser radiation, and bubbling did not have the effect of increasing the germination rate and germination energy of the seeds, but they did not reduce their viability, the results were at the level of the control sample. However, the use of bubbling after cryopreservation is not recommended, because all varieties showed a significantly significant decrease in the viability of seeds of the studied varieties. For Tugai seeds, we do not recommend using laser radiation as a pre-sowing treatment.

3. It has been proven that seeds of Alina and Nika varieties can be stored at ultra-low temperatures without loss of viability for up to 9 months, while short-term storage of no more than 3 months is recommended for Tugai and Novosibirskaya 84 varieties due to a decrease in germination with longer cryopreservation.

4. The analysis of the feed value showed the high nutritional value of the feed of varieties of Sudan grass grown in Karaganda. Despite the fact that the indicators

varied over the years, the exchange energy and feed unit were within the normal range, which indicates the high nutritional qualities of the feed. An analysis of the crop structure of Sudanese grass during the harvest period showed that the data in the cryopreservation variant on plant height was significantly higher than in the control. The experimental results indicate that cryopreservation had a positive effect on the seeds of Sudanese grass in the experimental area, resulting in more seeds than in the control.

5. The study of the anatomical structure of Sudan grass showed that between the control variants and the variant after cryopreservation, there are significantly significant differences in the size of root xylem cells, in the size of epidermal cells, parenchyma and stem xylem, which explains why plants after cryopreservation have a height significantly higher than the control. This is ensured by large xylem vessels, through which nutrients are transported faster and better through the plant, thereby ensuring good growth in height.

Implementation of work results.

The research results have been introduced into the educational and scientific process of the Laboratory of Biotechnology and Ecomonitoring of the E.A. Buketov KarNRU "Recommendations for cryopreservation of seed material" (Appendix B), as well as into the educational process of the Institute of Biology and Biotechnology of the Altai State University for teaching the disciplines "Big Practical Botany", "Reproductive Biology", "Anatomy and morphology of plants" for students of the direction 06.03.01 Biology (bachelor's degree), 06.04.01 Biology (Master's degree) (Appendix B)

The connection of this work with research projects.

The dissertation work was carried out within the framework of the grant project of the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan No. AP09259548 "Cryopreservation of seed material of wild and medicinal plants and the organization of a bank for short- and long-term storage" (2021-2023).

Personal contribution of the author. In obtaining scientific results, the author personally participated in the entire complex of research over the course of 6 years (2020-2026). The planning of a scientific experiment, the preparation of experimental plots, the laying of field and laboratory experiments on cryopreservation of Sudan grass seeds, anatomical sections of plants, the collection and statistical analysis of data, the generalization and scientific justification of the results were carried out by the author personally. The main provisions submitted for defense, conclusions were personally formulated, and the main sections of the dissertation research were written. Personally processed the data for writing and publishing scientific articles. The personal contribution of the author was 92%.

The volume and structure of the dissertation. The thesis includes an introduction, 5 main sections, a conclusion, a list of sources used and an appendix. The dissertation is presented on 150 pages, consists of 5 chapters, includes 67 figures,

11 tables, 8 appendices. The list of sources used consists of 189, including 43 in a foreign language.

Approbation of the work.

The results of the dissertation research were discussed and reported at:

1) international scientific and practical conference "Independence of Kazakhstan: aspects of biodiversity conservation" dedicated to the 80th anniversary of Doctor of Biological Sciences, Professor, Honorary Member of the National Academy of Sciences of the Republic of Kazakhstan, Academician Kaznayen Mukhitdinov Nashtay Mukhitdinovich international scientific and practical conference "Independence of Kazakhstan: aspects of biodiversity conservation" dedicated to the 80th anniversary of Doctor of Biological Sciences, Professor Honorary member of the National Academy of Sciences of the Republic of Kazakhstan, Academician of the Kazakh Academy of Sciences Mukhitdinov Nashtai Mukhitdinovich (Almaty, 2021 - November 26);

2) The International Scientific and Theoretical Conference "Food Safety: national and global aspects" (Samarkand, Uzbekistan, October 15-16, 2021), 3) the XV International Scientific and Practical Conference (Karaganda, January 20-21, 2023).

The results of the dissertation research were annually heard at meetings of the Department of Physiology of the Faculty of Biology and Geography of the E.A. Buketov Karaganda University. Based on the dissertation materials, 5 publications were published, including 1 article in the scientific journal "Research on Crops", which is part of the Scopus scientometric database (38%), 3 articles in journals recommended by the Committee for Quality Assurance in Science and Higher Education of the Republic of Kazakhstan "Bulletin of the Karaganda University, series biology" practical recommendations on cryopreservation of seed material of Sudanese grass have also been developed (Appendix A).