

## ABSTRACT

dissertation for the degree of Doctor of Philosophy (PhD)  
6D060700 - Biology

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### **Cryogenic storage and development of methods for short-term and long-term storage of *Chamomilla recutita* seed varieties**

**General characterisation of the work.** The dissertation work is devoted to the study of the conditions of cryopreservation of seed material of *Chamomilla recutita* varieties and the development of algorithms for long-term and short-term storage.

**Relevance of the topic.** The most valuable and irreplaceable natural resource are plants, especially medicinal plants, which have a wide application both in official medicine and in folk medicine. In modern medicine nowadays special attention is paid to medicines made from various types of medicinal plants. High interest in medicinal plants is explained by their high pharmacological activity. Preparations based on medicinal herbs are easily assimilated, not cumulated and easily excreted from the body, practically have no toxic effects, compared to drugs of synthetic nature.

The development of production of medicinal raw materials is relevant in Kazakhstan in light of the provision of local raw materials to the pharmaceutical industry.

In the flora of the republic there are about 6000 species of vascular plants, a quarter of which exhibit medicinal properties. In the GF RK 132 taxa are included, however, about 30% are not found in the local flora. It should be noted that Kazakhstan has small reserves of several species of medicinal plants, but they are insufficient for full provision of raw materials.

In medicinal plant breeding one of the important directions is seed production and creation of sustainable stocks of plant seeds. It is well known that seeds of most medicinal plants quickly lose their germination within 2-5 years and are stored for a limited time. Traditional methods of seed preservation at temperatures ranging from 0-+5 to -15°C are not fully capable of maintaining full viability for a long period. Since during storage physical and physiological changes occur (loss of moisture, defeat by fungi, bacteria, pests, accumulation of decay products, etc.). Therefore, the use of storage at liquefied nitrogen temperature (-196°C) appears to be a more promising direction for seed production of medicinal plants and conservation of their genetic resources.

Cryogenic storage makes it possible to store plant seeds for a long period of time, not only with preservation of vital processes, but also with increased viability. In the future, seeds can be stored for an unlimited period of time.

In Kazakhstan, the method of preservation of seeds of medicinal crops in liquid nitrogen is little tested, mainly studies are aimed at freezing meristems, pollen grains, buds, cuttings, etc.

As it was noted earlier that the process of seed cryopreservation has been studied for a long time, but the application of this method has been tested on a small number of medicinal crops, so we were tasked to determine the conditions of cryopreservation of seed material of *Chamomilla recutita* varieties and develop an algorithm for long-term and short-term storage.

Thus, the implementation of scientific research on the development of the basis for short-term and long-term storage of *Chamomilla recutita* seeds using cryopreservation methods is currently an important and relevant direction.

**Purpose of the research:** to study the effect of ultra-low temperatures on seed material of *Chamomilla recutita* varieties and to develop methods of long-term and short-term storage.

**Objectives of the study:**

1 Determination of seed viability depending on storage period and study of morphometric and anatomical parameters of seed material of chamomile varieties before and after freezing in liquid nitrogen

2. Optimisation of conditions of freezing and thawing of seed material of chamomile varieties after cryopreservation;

3. study of the influence of physical factors on the viability of seed material of varieties of chamomile apothecary and selection of optimal concentrations of protectors in cryopreservation.

4 Determination of quantitative accumulation of essential oils of chamomile apothecary varieties "Karaganda" and "Podmoskovnaya" after cryogenic storage

5. Development of algorithms for cryopreservation of seeds of chamomile varieties.

**The objects of the study** are seeds of chamomile apothecary varieties "Karaganda", "Podmoskovnaya", "Aibolit" and "Stary lekar". The seeds were collected in the nursery of the International Scientific and Production Centre "Phytochemistry" and also received by delectus from the Altai Botanical Garden.

**Research methods:** assessment of seed germination and germination biology, cryopreservation of seeds by direct immersion in Dewar vessels with liquid nitrogen, use of two methods of thawing (fast and slow thawing), anatomical study of seed structure and seedlings by pressing preparations, use of scanning electron microscope (SEM microscopy), determination of quantitative accumulation of essential oil by water distillation. Statistical processing and graphical visualisation of the obtained data were carried out using Microsoft Excel software and RStudio environment.

**Scientific novelty of the study**

1. The viability of seeds of 4 varieties of chamomile apothecary depending on the conditions of cryopreservation was investigated for the first time, anatomomorphometric indices of seedlings were studied;

2. The effect of cryoprotectors on germination and germination energy of seeds of chamomile varieties was revealed for the first time, which allowed to select the optimal types and concentrations of cryoprotectors.

3. The influence of pre-sowing methods of seed material treatment of chamomile varieties using physical factors was studied for the first time.

4. The quantitative accumulation of essential oil in aboveground organs of two varieties of chamomile apothecary, its component composition after storage of seeds in liquid nitrogen was revealed for the first time.

**Theoretical and practical significance of the results.** The study of the conditions of cryopreservation of seeds of varieties of chamomile apothecary showed that the optimal container for cryopreservation is plastic cryotubes.

It was determined that a slow thawing method should be used in the cryopreservation of chamomile seeds.

It is recommended to use PVS2 solution, highly concentrated sucrose, glucose, fructose and DMSO, also low concentrated propylene glycol as cryoprotectants.

It was determined that the anatomo-morphological structure of seeds and seedlings of all 4 varieties of chamomile apothecary does not change after exposure to ultra-low temperatures.

It was proved that long-term cryogenic storage of chamomile apothecary seeds increased the germination of 3 varieties.

It was revealed that after cryogenic storage, pre-sowing treatment of seeds with physical activation factors increases germination and germination energy of the studied varieties.

It was found that the content of essential oil and some components in the herb of chamomile pharmacy varieties "Karaganda" and "Podmoskovnaya" increases after cryopreservation of seeds

The developed algorithm of cryopreservation and methodological recommendations allowed to introduce the seeds of chamomile apothecary 4 varieties into the cryogenic bank of medicinal plants of the research park of biotechnology and ecomonitoring of E.A. Buketov BGF KarU".

The results of scientific research have been implemented in practical activities and educational process.

**The main points put forward for defence:**

1. storing chamomile seeds by the traditional method for 3 years reduces laboratory germination.

2. Optimisation of conditions for freezing of chamomile apothecary seeds showed that such varieties as Aibolit, Karaganda and Staryy Lekar positively reacted to long-term storage in liquid nitrogen. For seeds of Podmoskovskaya variety it is better to use short-term cryopreservation. When optimising seed thawing conditions, it was found that slow thawing is the most suitable thawing method for Podmoskovnaya, Aibolit and Karaganda varieties, while fast thawing is the most suitable for Stary Lekar.

3. During ultra-low freezing of chamomile seeds, highly concentrated cryoprotectants such as sucrose, glucose, fructose, DMSO and low-concentration propylene glycol, as well as cryoprotectant solution PVS2 should be used to prevent the formation of damaging ice crystals inside the cells.

4. Ultra-low temperature increases the quantitative accumulation of essential oils in the above-ground part of chamomile apothecary.

#### **Key Findings:**

1. It was found that the storage of seeds of varieties of *Chamomilla recutita* by the traditional method for 3 years shows a decrease in laboratory germination, especially in varieties "Stary lekar" and "Podmoskovnaya". Scanning electron microscopy, anatomy of seeds and seedlings showed that after cryopreservation seed coat of the studied varieties "Karaganda", "Podmoskovnaya", "Aibolit" and "Stary Lekar" are not damaged, significant morphological changes in the structure of the surface of the seed coat are not observed, the integrity of the seeds is preserved. Also no changes in the anatomical structures of seeds and seedlings (seed leaves, hypocotyl, root tip) after cryopreservation of the studied varieties of *Chamomilla recutita* are not observed.

2 Determination of freezing conditions of seeds of *Chamomilla recutita* varieties showed that varieties "Karaganda", "Aibolit" and "Stary lekar" reacted positively to long-term storage in liquid nitrogen (6 months). When cryopreserving seeds of Podmoskovskaya variety, it is recommended to use short-term ultra-deep freezing. The optimal thawing mode after cryopreservation is slow thawing at room temperature for "Karaganda", "Aibolit" and "Podmoskovnaya" varieties; for "Stary Lekar" variety, it is recommended to use fast thawing in a water bath at +40°C.

3. When studying the effect of physical factors on the viability of seeds of *Chamomilla recutita* after cryopreservation, a positive stimulating effect of barbotage and magnetic field on varieties "Karaganda" and "Aibolit" was revealed. For varieties "Podmoskovskaya" and "Stary lekar" physical activation factors were ineffective. Selection of optimal concentrations and types of cryoprotectors showed that the most effective for all varieties, except for 'Aibolit', are: from non-penetrating cryoprotectors - glucose (40%), sucrose (40%) and fructose (20%, 25%); from penetrating - highly concentrated 15% DMSO and propylene glycol (5%, 10%). Also, PVS2 solution had a positive effect as a cryoprotector during cryogenic storage of seeds of "Podmoskovnaya" and "Karaganda" varieties.

4 The positive effect of ultra-low temperatures on the chemical composition and quantitative accumulation of essential oil in the above-ground part of *Chamomilla recutita* was found. Thus, in the plant grown from cryopreserved seeds the accumulation of essential oil increases and the percentage content of sesquiterpene class components and substances of the ether group increases.

5. Based on the results of these studies, an algorithm for cryopreservation of seed material of 4 varieties of *Chamomilla recutita* was developed.

#### **Implementation of the results of the work.**

The results of the research are implemented in the educational process of the Faculty of Biology and Geography of NAO "Karaganda University named after Academician E.A. Buketov" in the disciplines of botany and cryobiology, in the research park of biotechnology and ecomonitoring to perform work on cryobiology, as well as implemented in the scientific process to organise the work of the seed bank of plants of the Altai Botanical Garden.

#### **Relation of this paper to research projects.**

The dissertation work was carried out within the framework of the implementation of the grant project of the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan № AR09259548 "Cryopreservation of seed material of wild and medicinal plants and the organisation of the bank of short-term and long-term storage" (2021-2023).

#### **Author's personal contribution.**

The author personally developed the programme of the dissertation research, conducted the experimental part of the work, and carried out research on cryopreservation of seed material. The author was personally engaged in the collection of initial data, statistical processing and analyses of the obtained results. The main provisions, conclusions were formulated, the main sections of the dissertation research were written. Scientific articles were independently prepared and published. The author's personal contribution was 90%.

**The scope and structure of the thesis.** The thesis includes an introduction, 6 main sections, conclusion, list of used sources and appendices. The volume of the thesis is 151 pages, the work is illustrated with 52 figures, 10 tables. The list of used sources includes 235.

#### **Approbation of the work.**

The results of the dissertation research were discussed and reported at the international scientific conference "Actual problems of biology and ecology" (Karaganda, 16-17 November 2018); All-Russian scientific conference with international participation dedicated to the 120th anniversary of N.V. Tsitsin "The heritage of Academician N.V. Tsitsin. Current state and prospects of development" (Moscow, 8-11 July 2019); VI All-Russian Conference with international participation "EcoBioTech 2019" (Ufa, 1-4 October 2019); V International Scientific and Practical Conference of students and young scientists "Methodology, theory and practice of modern biology" (Kostanai, 13 March 2020.); international scientific-practical conference "Medicinal plant growing: from the experience of the past to modern technologies" (Poltava, 29-30 June 2020); international scientific conference of young scientists "Modern trends in the development of health-saving technologies" (Moscow, 2021, December 16-17); XV international scientific-practical conference "Actual problems of ecology" (Karaganda, 20-21 January 2023), Republican scientific-practical conference (with international participation), timed to the 80th anniversary of the scientist-teacher, professor of the Department of Botany Akhmetzhanova Aitbala Ibzhanovna "Actual problems of biology and ecology". The results of the dissertation

research were published in the scientific journal, included in the Scopus scientific database "Research on Crops", in the journals recommended by the SHEQAC RK - "Bulletin of the Karaganda University, series biology". The monograph on cryopreservation of seed material of medicinal plants was published and practical recommendations were developed.