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Biological Study *Scabiosa ochroleuca* Seeds Germination and Development of Methods for a Long-Term Storage

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Abstract: The organization of a storage system for seeds of medicinal plants is an important task of introduction. Our study aimed to identify biological features during the germination of *Scabiosa ochroleuca* seeds and optimize the conditions for their storage and germination activation. The paper presents the results of assessing the viability of seeds of *S. ochroleuca* depending on the origin, size, storage time, the use of pre-sowing treatment with epin, potassium humate, the use of barbotage and stratification; the conditions of cryopreservation in liquid nitrogen have been worked out. The results showed that germination capacity and energy depended on origin and size. The best indicators were noted for large and completed seeds collected in the Buyratau Mountains. During storage, a gradual decrease in germination was noticed; the loss of viability was noted in the 4th year of storage under normal conditions. Stratification can be used for 3 months to activate germination. To organize a long-term storage system, we recommend storing *S. ochroleuca* seeds in liquid nitrogen in plastic containers, using glycerol cryoprotectant at a concentration of 40%.

Keywords: *Scabiosa ochroleuca*, Seed Material, Germination Capacity, Germination Energy, Storage Condition, Pre-Sowing Treatment, Cryopreservation, Cryoprotectants

Introduction

The Scabiosa genus (*Scabiosa* L.) of the teasel family (*Dipsacaceae*) has about 100 species (618 botanical names), mainly distributed in the Mediterranean, 8 species of which can be found in Kazakhstan (Pavlov, 1965; Baitenov, 2001).

The introduction of new types of medicinal plants into practical use makes it possible to expand the list of medicinal raw materials and create phytopreparations based on local raw materials (WHO, 2005; Chen *et al*., 2016; Miranda, 2021). About 5500 species of vascular plants grow in Kazakhstan, 1200 species of which are used in traditional medicine, but only about 130 species are used in official medicine (Tulegenova, 2008).

Plants of the genus *Scabiosa* are currently used in alternative medicine as a vulnerary product, for hemorrhoids, skin diseases (skin rashes, scabies, and warts), eyes, women's diseases, respiratory diseases, coughs, diseases of liver and bladder, inflammation of the kidneys and urinary tract, venereal diseases, etc. (Krupennikova and Fedoseeva, 2008; Endova and Ancupova, 2009; Al-Qudah, 2016; Malyutina *et al*., 2017; Pinto *et al*., 2018; Rahmouni *et al*, 2018; Maroyi, 2019; Zhunusova and Akhmetova, 2020).

A prospective species for future study is *Scabiosa ochroleuca* L., a biennial or perennial herbal plant, which is widespread in all regions of Kazakhstan (Baitenov, 2001). The period of flowering is June-August, and fruiting-is in August-September. The general area includes Western Europe, Caucasus, the Caspian region, Western China, Mongolia, and Western and Eastern Siberia. The specie grows in the steppes, meadows, along the slopes of hills, in the mountains, and in shrub thickets.

Experimental studies have established that biologically active substances from *the S. ochroleuca* herb cause lysis of malignant tumors, the water-alcohol

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tincture of the seeds exhibits anti-amoebic activity and the polyphenol complex has antioxidant properties (Endova, 2004; Zhunussova *et al*., 2017a,b; Drozdova and Minakova, 2018; Mukanova *et al*., 2019; Zhunussova, 2019; Mulanova, 2019; Zhunussova *et al*., 2022).

There is a prospect for utilizing this species as a source of medicinal raw materials. Cultivation of *S.ochroleuca* on a larger scale is needed as it has limited resources in nature (Ishmuratova *et al*., 2020). This study on the biology of germination of plants with the development of methods for seed storage is required.

The purpose of this study is to identify biological features during the germination of *S. ochroleuca* seeds and to optimize their storage conditions.

Materials and Methods

Seed Material

The seed materials of *S. ochroleuca* were collected on the territory of Central Kazakhstan (Table 1).

Germination Analysis

The seeds were dried, packed in paper bags, and stored in a refrigerator. Seed morphology was studied on an MBS-1 binocular microscope under laboratory conditions at a magnification of 40-80 times; photographs were taken on a Levenhuk microscope. The weight of seed samples was estimated by weighing in 5 repetitions of 50 pieces on an analytical balance Shimadzu AY 220, recalculating for 1000 pieces. Humidity was analyzed on an OHAUS MB23 moisture meter.

All seed samples were separated into three groups by length: Large (3.0-3.5 mm), medium (2.5-2.9 mm), and small (less than 2.5 mm) using a sieve. All experiments were carried out in 4-fold repetitions.

To assess the germination of seeds, they were germinated 50 pieces in three repetitions on moistened 2-layer filter paper in Petri dishes in a climatic chamber at a temperature of +24°C. For experiments, seeds were not specially selected, only damaged ones with a changed color or empty were rejected. Seed germination capacity (on day 20) and germination energy (on day 7) were assessed according to methodical guidelines (Copeland and McDonald, 1999; IRST, 2019).

We studied the dependence of germination capacity rates on the place of the collection; seed size and weight; germination in the light and the dark; storage periods. Experiments were carried out to activate germination capacity using physical (stratification, barbotage) and chemical methods (Erohin and Cukanova, 2014; Araujo *et al*., 2016; Siddique and Kumar, 2018; Aksenovskij *et al*., 2019). Physical treatment was carried out by (i) barbotage in an aqueous medium with compressed air for 24 h; and (ii) stratification within 3 months. Chemical treatment was carried out by soaking the seeds for 12 h in solutions of growth regulators: (i) Potassium humate 0.01%; and (ii) epin 0.001%.

To establish the protocol for long-term storage in liquid nitrogen, experiments were carried out to optimize the conditions of cryopreservation. The seed material was placed in liquid nitrogen in Dewar's vessels SDS 20 Cryo Mash (Panis and Lambardi, 2006; Romadanova, 2017). The following freezing options were tested: (1) Two types of containers (Falcon plastic cryovials; metalized Zip-lock bags); (2) two types of defrosting (fast - in a water bath at +45ºC, slow - at room temperature +22°C; and (3) application of cryoprotectants (sucrose, glucose, propylene glycol, glycerin, DMSO), water solutions. Reagents manufactured by Sigma-Aldrich.

Statistical Processing

Mathematical data processing and compilation of algorithms for calculating the main statistical series of observations were carried out according to the methods of (Lakin, 1990) using the Statgraphics Centurion XV.I statistical software for Windows. The studied variants from different geographical points were compared with each other to choose the best place to organize the collection of seeds for placement in a cryobank. Seed treatment experiments were compared with control values.

Table 1. Places of *Scabiosa ochroleuca* seeds collection on the territory of Central Kazakhstan

#	Sampling location	Date of collection	GPS-coordinates
1	Karkaraly Mountains, Karkaraly district, Karaganda region	23.08.2017	N 49º23’693” E 75º52’069” 465 m above sea level
2	Outskirts of Karagaily the village, Karkaraly district, Karaganda region	23.08.2017	N 49º34’216” E 75º72’359” 318 m above sea level
3	National State Natural Park “Buyratau”, Osakarovsky district, Karaganda region	11.09.2017	N 51º12’578” E 73º60’034” 408 m above sea level

Results and Discussion

Analysis of seed Germination Depending on Morphology and Weight of S. ochroleuca

The fruit of *S. ochroleuca* is an achene (Fig. 1) with a bell-shaped veil, conical in shape, narrowed nose, and obtuse; the surface is rough, covered with numerous deep ribs.

Studies have shown that seed material of different geographical origins differs in size and weight (Table 2). As can be seen from the data, the maximum length was 3.4 mm for the seeds from the Karkaraly Mountains, while in terms of thickness, the maximum values were obtained for seeds from the outskirts of Karagaily village-1.8 mm, no significant difference was found between the length indicators.

There were significant differences in seed length, width, and weight between the variants collected from different places of growth. The maximum seed length (3.4 mm) and weight (0.32 g) were recorded for seeds from the Karkaraly Mountains. The smallest size (length 2.6 mm; width 1.5 mm) and weight (0.16 g) were noted for seeds from Buyratau Mountains.

However, the size and weight of the seeds do not yet reflect the quality indicators, so we have determined the germination capacity and germination energy. A statistically significant difference in seed germination capacity was noted, the germination energy indicator was significantly lower for seeds from the Karkaraly Mountains. There were statistically significant differences between germination capacity and germination energy from seeds from the Buyratau Mountains and the outskirts of Karagaily village.

Thus, it can be concluded that the seed material of *S. ochroleuca* differs in size and weight depending on the place of growth. So, the largest seeds are formed in the mountains of Karkaraly, and the smallest-are in the mountains of Buyratau. This aspect can be explained by the fact that more mesophytic conditions (more precipitates) are observed in the Karkaraly Mountains (Vilesov *et al*., 2009), which leads to a good development of plant morphology. In the Buyratau Mountains, the conditions are more xerophytic, so the seed size is lower. However, the data on germination and germination energy was higher in the Buyratau Mountains.

The seed material of *S. ochroleuca* is of different quality, which is explained by the difference in the timing of setting and ripening on the 1st inflorescence. We analyzed the germination capacity of seeds depending on their size (Table 3).

The results showed that the maximum germination capacity was recorded for large seeds, that is, when selecting seeds for storage, it is necessary to make a selection in favor of larger ones. Statistically significant differences were noted in terms of the germination capacity of seed fractions from the Buyratau Mountains, while for seeds from the outskirts of Karagaily village and Karkaraly Mountains, significant differences in germination capacity were noted only for the fraction of small seeds compare with large and medium seeds.

Seed Germination Depending on Period of Storage

An assessment of the dynamics of germination capacity during storage showed (for non-separated seeds) that there is an increase in germination capacity after 3 months, which can be explained by the effect of ripening (Finch-Savage and Leubner-Metzger, 2006), after which a gradual decrease is observed (Fig. 2).

Seed Germination Depending on Applying Physical and Chemical Methods of Pre-Sowing Treatment

To increase the germination capacity of seeds after 3 years of storage, we used the methods of pre-sowing treatment with growth regulators and the use of barbotage and stratification (Fig. 3).

According to the results of experiments with the use of epin, an increase in germination capacity by 1.8%, potassium humate - by 16.3%, and barbotage - by 25.6% is observed.

The maximum effect was achieved during stratification, while germination capacity increased by 35.5%, and germination energy by 39.1%.

Cryopreservation of Seed Materials of S. ochroleuca

In the next experiment, we assessed the effect of packaging and defrosting conditions on the germination capacity and germination energy of *S. ochroleuca* seeds (Fig. 4).

The results showed that the germination capacity of freshly harvested seeds of *S. ochroleuca* was extremely low -8.8%. Seed germination was observed 3-4 days after sowing. Before germination, the seeds swell, increasing in size by 20-25%. After short-term freezing in liquid nitrogen, the best indicators of germination capacity and germination energy of *S. ochroleuca* seeds were obtained in the variant of using plastic containers -55.2%.

However, the germination capacity and germination energy of *S. ochroleuca* seeds in all freezing options turned out to be statistically significantly lower than the control values by 3.5-38.8%.

Since the previous experiments showed that for pale yellow Scabiosa the results of freezing in liquid nitrogen without the use of cryoprotectants turned out to be lower than seeds stored under normal conditions, we set up a series of experiments on the use of various cryoprotectants (Fig. 5).

Experiments have shown that the use of some cryoprotectants has a positive effect on increasing the germination capacity and germination energy of *S. ochroleuca* seeds. The best option for *S. ochroleuca* seeds is the use of glycerol at a concentration of 40%, at which a statistically significant increase in germination capacity by 5.7% was noted.

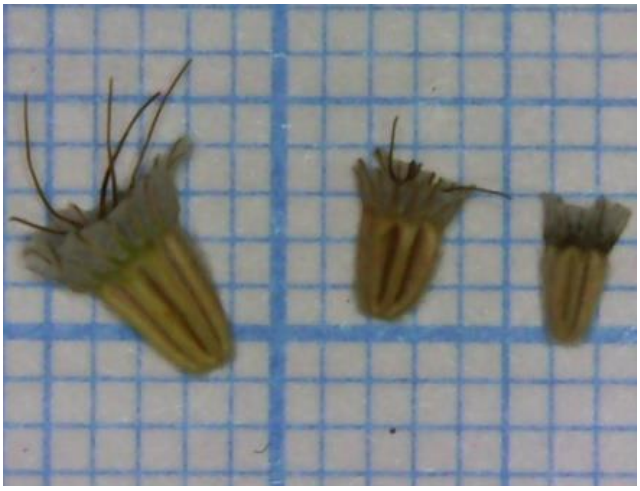

Fig. 1: Appearance of *Scabiosa ochroleuca* seeds

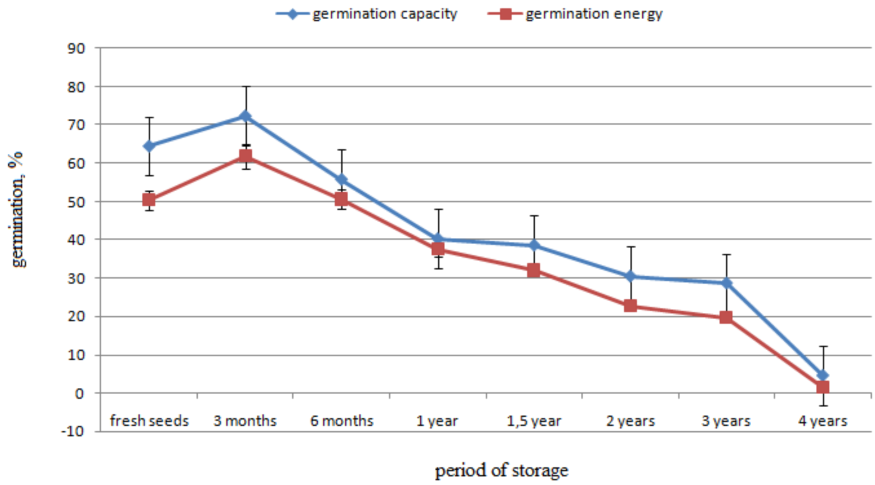

Fig. 2: Dynamics of germination capacity and germination energy of *Scabiosa ochroleuca* seeds during storage

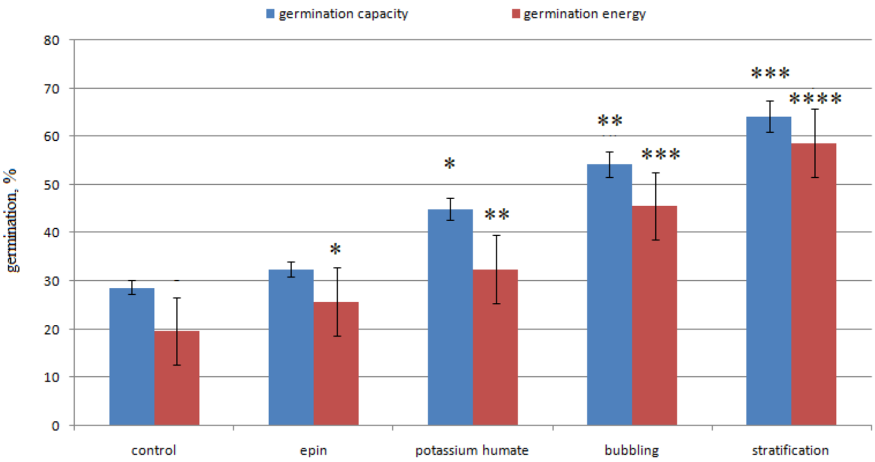

Fig. 3: Germination capacity and germination energy of *Scabiosa ochroleuca* seeds depending on the method of pre-sowing treatment; * show statistically significant differences (at the accuracy of the experiment P<5)

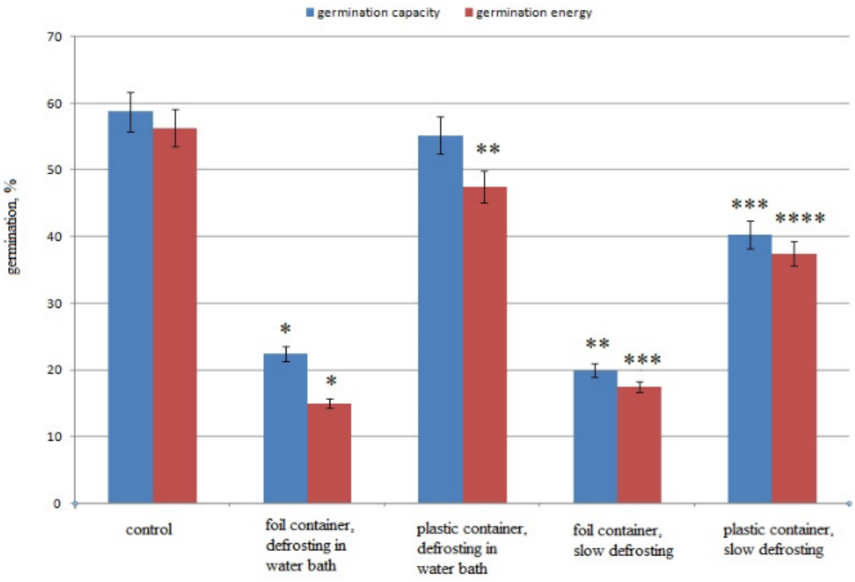

Fig. 4: Germination capacity and germination energy of *Scabiosa ochroleuca* seeds depending on the type of container and the conditions for defrosting the method; * show statistically significant differences (at the accuracy of the experiment P<5)

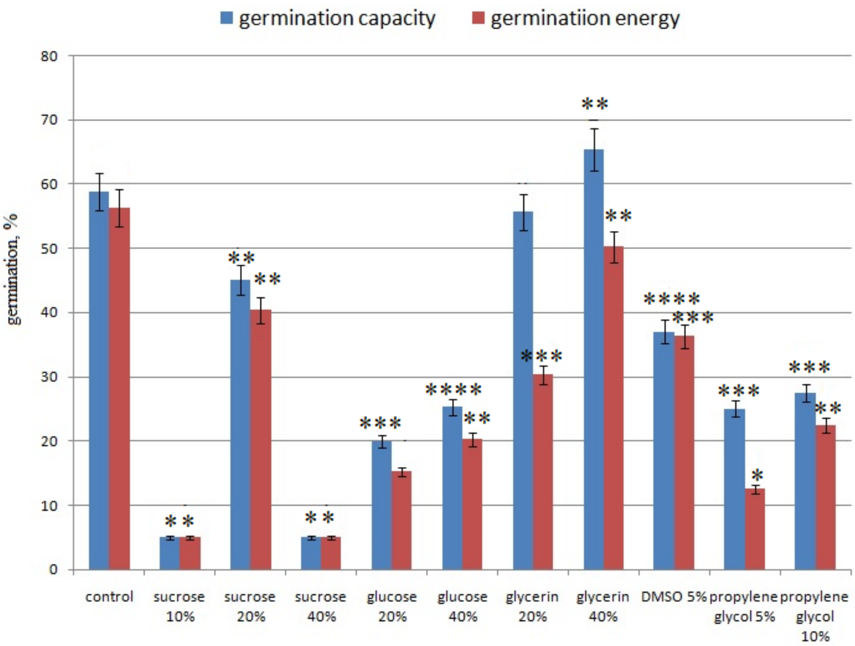

Fig. 5: Germination capacity and germination energy of *Scabiosa ochroleuca* seeds depending on the type and concentration of the cryoprotectant; * show statistically significant differences (at the accuracy of the experiment P<5)

Table 2: Morphological parameters of *Scabiosa ochroleuca* seeds depending on the origin

	Origin of seeds		
Parameters	Buyratau Mountains	Outskirts of Karagaily village	Karkaraly Mountains
Average seed length (mm)	2.6±0.20	2.7±0.10	3.40 ±0.40*
Average seed (width, mm)	1.5±0.06	1.8±0.04*	1.70 ±0.05*
Weight 1000 (items, g)	0.16±0.01	0.21±0.01*	0.32±0.02**
Germination (capacity, %)	64.5±2.60	61.0±2.80	50.10±2.10*
Germination (energy, %)	50.4±0.80	52.9±1.80	41.00±0.90*

Note: * - show statistically significant differences in indicators P<5

Table 3: Germination capacity and germination energy of *Scabiosa ochroleuca* seeds depending on size

Origin of seeds	Fraction of seeds	Germination capacity, %	Germination energy, %
Buyratau Mountains	Large	90.5±3.5	80.3±3.9
	Medium	62.4±2.8*	51.2±2.5*
	Small	26.4±0.5**	20.1±0.7**
Outskirts of Karagaily village	Large	60.2±3.0	50.1±1.8
	Medium	61.4±3.4	52.0±1.6
	Small	28.5±0.8*	12.4±0.6*
Karkaraly Mountains	Large	46.2±1.1	38.0±0.8
	Medium	45.5±1.0	40.1±1.1
	Small	14.0±0.5*	8.5±0.3*

Note: * show statistically significant differences in indicators P<5

Conclusion

Thus, we have studied the biology of germination of *S. ochroleuca* seeds depending on the origin, size, and storage period. It was determined that the best performance was noted for seeds collected in more arid conditions, with the maximum size and weight. During storage, a decrease in viability is observed, as a result of which, in the 4th year, under normal conditions, the seeds practically do not germinate. To activate germination capacity, stratification for 3 months can be used.

To organize a long-term storage system, *S. ochroleuca* seeds can be stored in liquid nitrogen in a plastic container using glycerol cryoprotectant at a concentration of 40%.

The information obtained allows us to develop an algorithm for the preparation and organization of long-term storage of *S. ochroleuca* seeds at supercritical low temperatures.

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Author's Contributions

Sayagul S. Tyrzhanova: Concept, manuscript writing, critical revision of the manuscript, assessment of seed germination, data collection.

Margarita Yu. Ishmuratova: Analysis, manuscript writing, data interpretation, identification of species, collecting of seed material, final approval.

Sauran N. Atikeeva: Data interpretation, collection of seed material, cryopreservation of seeds.

Ainur Sh. Kydyrmoldina: Assessment of seed germination using physical methods pre-sowing treatment.

Meruyert M. Malik: Analysis of morphological indicators of seeds, statistic analysis, manuscript writing.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all co-authors have read and approved the manuscript and that no ethical issues are involved.

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