

Study of the Cryopreservation Conditions for seed Materials of *Chartolepis intermedia* Boiss.

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Abstract. For the first time, the depending of germination rate and energy of germination of *Chartolepis intermedia* seeds, collected in the wild of Karaganda region, from morphology of seeds and conditions of cryopreservation is investigated. The maximum results for viability are fixed for dark-colored average or large seeds. The best results are determined for variant of cryopreservation in plastic container with future defrosting at the room temperature. For the increasing parameters of seed germination we recommended to freeze *Chartolepis intermedia* seeds with using cryoprotector sucrose in concentration 20%. As the results of the study, we developed algorithm of cryopreservation of *Chartolepis intermedia* seeds in liquid nitrogen.

1 Introduction

Republic of Kazakhstan has a rich diversity of medicinal plants: from 5500 species of vascular plants [1] accordantly 1200 species have medicinal properties [2]. Not all plants are suitable for collected in nature due their rarity, lack of vegetative resources and remoteness of natural populations. One the optimal decision is introduction medicinal plants for future industrial cultivation n production of required volumes of raw material [3]. In process of introduction experiment is necessary to develop effective methods for the cryopreservation of seed materials of prospect herbs [4]. Present day the most optimal method for storage of gene pool is cryopreservation, which allows storing seed material for a long period without loss of viability [5, 6].

The purpose of the present study is to determine optimal methods for cryopreservation of seed material of perspective medicinal plant – *Chartolepis intermedia*.

2 Materials and methodology

2.1 Objects

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Chartolepis intermedia Boiss. is a perennial herbal plants from *Asteraceae* family, grows in alkaline (salty) and steppe meadows, in steppe declines, river floodplains, along streams, in the vicinity of settlements [1, 7]. Biological active compounds from areal part have antibacterial, antiviral and inflammatory activity [8], which has the potential for developing the new domestic phytopreparations [9].

Seed material for the study is collected in Spassky hills (Karaganda region; N 49,49106; E 73,23768), phase – heavy bearing.

2.2 Assessment of seed viability

Seeds of *Chartolepis intermedia* are frozen in the liquid nitrogen in the Dewar vessels using foil and plastic containers [10]. Assessment of germination rate and energy of germination are conducted on Petri dishes during 3 weeks [11]. For optimization storage conditions different defrosting methods (fast – on water bath, +40 °C; slow – at the room temperature, +24 °C) and cryoprotectors are evaluated.

3 Results

Seed of *Chartolepis intermedia* is mericarp, length 5-6 mm, width 1.6-2 mm, weight of 1000 seeds is 3.5-5 gram. They differ in size, color and weight, which depend on placement in flower baskets and time of ripening. The different variants showed different indicators of germination rate and energy of germination (Table 1).

Table 1. The viability of *Chartolepis intermedia* seeds on color and size

Variant of seed samples	Germination rate, %	Energy of germination, %
White small	2.5±0.04	2.2±0.05
Beige small	20.0±0.1	15.2±0.1
Brown average	67.5±2.5	42.5±0.8
Brown large	72.5±3.2	52.5±2.7

Results influence type of containers and method of defrosting on viability of *Chartolepis intermedia* seeds is presented in table 2, influence of cryoprotectors – in table 3.

Table 2. Germination of *Chartolepis intermedia* seeds depending on the type of containers and method of defrosting

Variant of experience	Germination rate, %	Energy of germination, %
Plastic tubes, slow defrosting	50.0±1.8	37.5±0.4
Foil package, slow defrosting	42.5±0.9	32.5±0.6
Plastic tubes, fast defrosting	47.5±0.8	27.5±0.5
Foil package, fast defrosting	45.0±0.6	28.3±0.4

Table 3. Germination of *Chartolepis intermedia* seeds depending on the type of containers and method of defrosting

Cryoprotector, concentration	Germination rate, %	Energy of germination, %
Sucrose, 5%	67.5±3.2	42.5±0.7
Sucrose, 10%	55.0±1.9	20.0±0.8
Sucrose, 15%	62.5±3.2	32.6±0.5
Sucrose, 20%	70.2±3.5	37.6±0.4
Glucose 5% + Glycerine 40%	62.5±2.5	27.6±0.3
Glucose 25% + Glycerine 40%	27.5±0.1	12.5±0.07
Glucose 45% + Glycerine 40%	45.0±0.8	20.3±0.1

4 Discussion

For *Chartolepis intermedia* seeds is note that germination dependence on size and. So, the best results are obtained for dark-colored seeds of average or large size (67.5-72.5%). There, for the laying seeds for the storage, it is necessary to take out a large fraction.

The results of freezing of *Chartolepis intermedia* seeds in liquid nitrogen showed that they are able to tolerate extra-critical low temperatures. Germination rate and energy of germination for seeds, frozen in plastic containers, were higher than in foil containers. In optimizing defrosting conditions, the best results are obtained for the option of freezing seeds in plastic containers and defrosting at the room temperature.

The use of cryoprotectors made it possible to establish positive results exceeding the control values for the 20% sucrose variant. So, germination rate was 70.2%, energy of germination – 37.6%. Based on the results of research, recommendations and an algorithm for freezing *Chartolepis intermedia* seeds in liquid nitrogen are developed.

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