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The cryoconservation of seed materials of *Serratula kirghisorum*

Seed germination and viability of seed material of *Serratula kirghisorum* of various methods of cryoconservation are studied; influence of a container, a method of freezing and defrosting, application of cryoprotectors is defined. The best indicators have been received at use of glycerin as cryoprotector, plastic test tubes. Thawing of seed material needed to be carried out quickly with application of a hot water bath. The biology of germination of seed material at various methods of cryodeposition is studied. Essential distinctions in biology germination of seeds after a cryopreservation aren't revealed. The sprout passed all phases of development; the sizes of the main parts of sprout had not considerable differences. After freezing in liquid nitrogen passing by a sprout of phases of development happened slightly quicker, on average for one day, besides sprouts were more viable and strong. All results testified about safety of a cryopreservation for a seed germ, and allowed to use this method for preservation of seed material during creation of a collection of endemic plants' seeds.

Key words: seed material, storage, cryoconservation, liquid nitrogen, endemic plant, *Serratula kirghisorum*.

The deep freezing of seeds is considered one of perspective methods of storage of plant genomes (until the temperature of liquid nitrogen), that theoretically allows to keep viability and genetic full value of seed material unlimited time. However, it is important to optimize conditions of introduction of a species to a collection as much as possible to save the available viability of seed material. The various factors exert impact on safety of viability of seeds: freezing speed, thawing speed, humidity of seeds, existence of a cryoprotectors and even container [1–3].

The method of deep freezing of seed materials of *Serratula kirghisorum* was not applied, the tolerance of seeds to storage at ultralow temperatures was not studied.

The aim of present research was the choice of optimum conditions of cryostorage of seed material of the endemic ecdysterone-containing plant.

Methodology

Object of research was the seed materials of *Serratula kirgisorum* Iljin, Asteraceae family. *Serratula kirgisorum* is perennial grassy plant, 10–40 cm high. Plant stalk is single, simple, direct, ridge, almost naked, with the leaf in the top part. Leaves from below are more pale, naked, only on a border with dense short eyelashes; radical and lower stem leaves and are oval or extended, short and petiole, almost lira-shaped, gear, seldom plumose and separate; stem leaves are sedentary, lira-shaped or plumose and separate; top leaves are often un-separable, lanceolate and linear, small by size. The basket is single; spathe is 12–15 mm long and 10–20 mm wide, greenish-golden, naked or a little ragged; external leaves are oval, middle leaves of flower basket are wide — lanceolate with tenon 1–3 mm, on the top are pale-brown, with streaks, internal leaves have on a top short trichomes. The receptacle is setaceous. This species grows on salty and clay-stone steppe of desert regions of Kazakhstan and in Mountains Altai, Tarbagatai and Dzhungar Alatau. Endemic of Kazkahstan [4–6].

Research of viability and energy of seed germination is carried out according to M.S.Zorina and S.P.Kabanov [7], M.V.Maltseva's methodical instructions [8].

In vitro seeds are couched in Petri's dishes in 4-fold frequency on 2 layers of the filter paper moistened with the distilled water. Petri's dishes with seed material placed in the climatic cell at a temperature +24 ^{0}C. For experiments seeds didn't select specially, rejected only damaged, with the changed coloring or empty.

Statistical processing of results was conducted by N.L.Udolskaya's technique [9].

Freezing of seeds was carried out by two ways. Seeds gradually cooled up to the temperature — 48–50 ^{0}C in the freezer Sanyo Medikal Freezer, the MDF model — U 442 (T), with a two-level method with an initial interval of 1–2 ^{0}C of half an hour up to the temperature of -30 ^{0}C. In the second step of cooling the speed of freezing was increased to 4–5 °C in half an hour and brought to temperature of -50 ^{0}C, after that seeds placed in big cryotanks on storage in nitrogen vapors at the temperature 183–185 °C [10]. Besides used fast freezing by immersion of seeds in various containers directly in liquid nitrogen with temperature — 196 °C, as a cryoprotector was used glycerin and 10 % water solution of sucrose.

Results and discussion

The series of experiments on freezing of seed material of the studied species has been carried out. Seeds of *Serratula kirghisorum* in various container: fabric sacks, plastic test tubes (cryotest tubes of the Nunc brand), envelopes from a foil, immersed in liquid nitrogen (-196 °C). Thawing of seeds was carried out in various ways — slowly, at the room temperature; quickly, on a water bath with a temperature of 80 °C; applied crops with a two-day delay after thawing on air.

Initial viability of seed material was 71,77±0,6 %, energy of germination was 36,06±0,7 %.

Literary data demonstrated that the container in which objects immerse in nitrogen exerts impact on safety of biological material. In experiment we used a foil, plastic test tubes and a container from fabric (Fig. 1). The best degree of safety of viability and energy of germination was shown by the seeds frozen in plastic test tubes — 75,75±2 %. Using of a container from a foil and fabric led to decrease in preservation of viability of seeds 56 % and 53 % accordantly, that was 78 % and 77 % of initial viability of seeds. Thus, for cryodeposition of seeds of *Serratula kirghisorum* we recommended to use plastic test tubes.

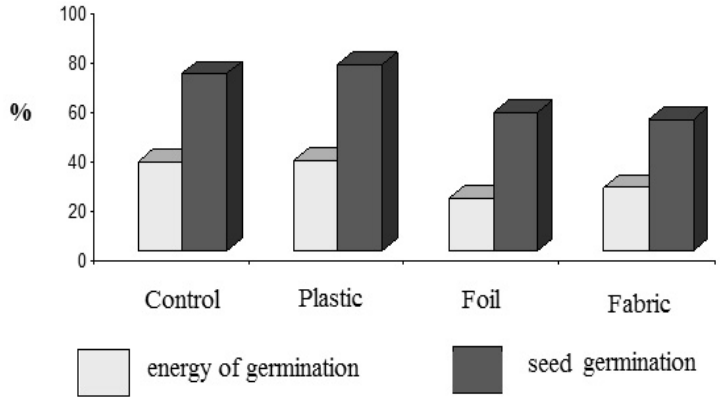


Figure 1. Dependence of preservation of viability of seeds of *Serratula kirghisorum* on the container used at fast freezing

At introduction of biological material in genetic bank it is necessary to optimize the mode of thawing of objects. We have studied degree of safety of viability of seeds of *Serratula kirghisorum* after fast freezing by immersion in liquid nitrogen and various ways of thawing — slow at the room temperature, fast on a water bath (Fig. 2).

It is defined that the best way of thawing for seeds of *Serratula kirghisorum* was fast defrosting on the water bath, viability at the same time has made 86,8 % that above, than in control variant for 15 %, and above variant with slow thawing for 10 %. In spite of the fact that the difference was not really considerable, we recommended to use for a cryopreservation of seeds of this species a plastic container and fast defrosting, because during long-term deposition it was important to keep viability of seed material as much as possible.

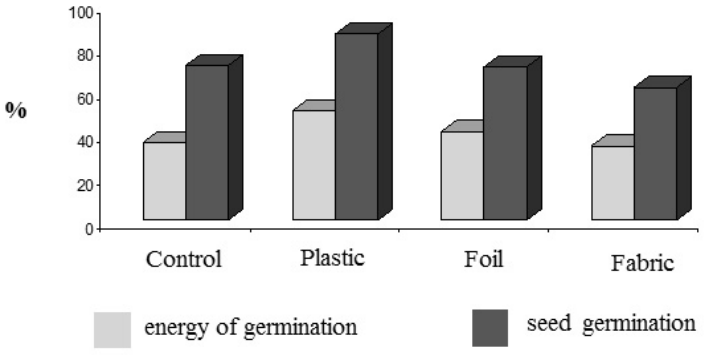
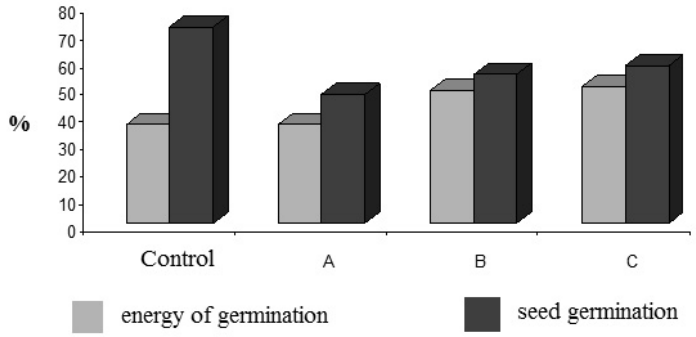


Figure 2. Preservation of viability of seeds of *Serratula kirghisorum* after a cryopreservation and fast thawing

During contacted our researches we have used the method of program freezing mastered at the All-Russian Research Institute of Plant Growing of N.I.Vavilov (Saint Petersburg). Seed material was exposed to two-level freezing — slowly to -30 °C and -50 °C, then quickly immersing in vapors of liquid nitrogen at a temperature of -183–185 °C. Defrosting of seeds was carried out slowly at the room temperature. The received results (Fig. 3) have shown that all three options of experiment have led to decrease in viability of seed material. However, the best of the received indicators were cooling to — 50 °C without immersion in nitrogen vapors. These results demonstrated that there was no full exit of intracellular free water and at deep freezing the ice crystals damaging a germ are formed.



A — Cooling to -30 °C, immersion in liquid nitrogen; B — Cooling to -50 °C, immersion in liquid nitrogen; C — Cooling to -50 °C

Figure 3. Preservation of indicators of germination of seeds of *Serratula kirghisorum* after two-level freezing

In spite of the fact that seeds of *Serratula kirghisorum* have low humidity and belong to orthodox group, extent of preservation of viability of seeds at fast freezing in cryoprotectors has been studied (Fig. 4, 5). Thawing was carried out by two ways — at the room temperature — slow and fast — on a hot water bath.

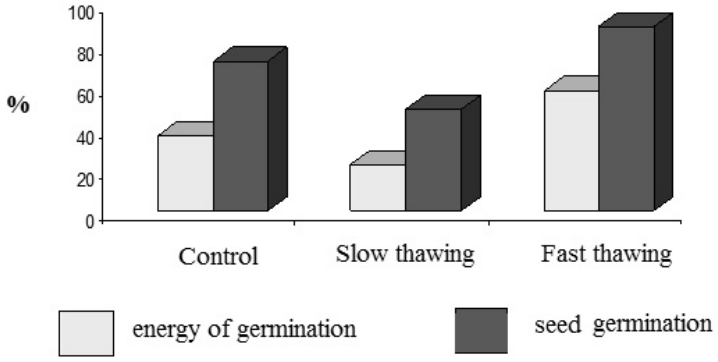


Figure 4. Influence of glycerin on preservation of survival of seeds at cryostorage

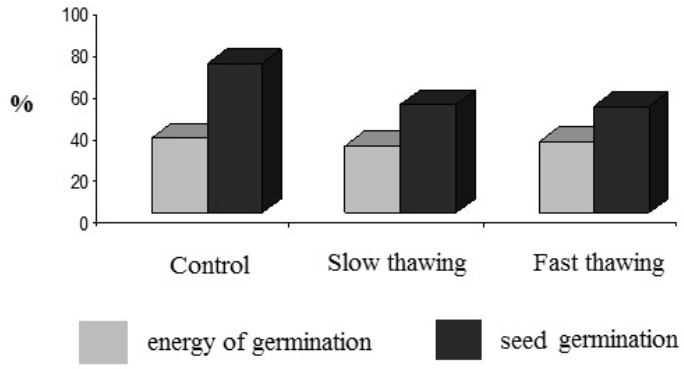


Figure 5. Influence of 10 % solution of sucrose on preservation of survival of seeds at cryostorage

Use of glycerin as a cryoprotector has considerably increased viability of seeds and seed germination. So, seed germination after fast thawing (cryoconservation in glycerin) was 88,25 %, that was 122 % from initial viability. After slow thawing growth indicators were decreased until 48,8 %, that one more time emphasized the fact about fast thawing in water bath of seed of *Serratula kirghisorum.* Using sucrose solution as acryoprotector with concentration of 10 % was not led to bigger preservation of viability of seeds. The solution of sucrose did not interfere for formation of ice which led to damage of a germ.

Dynamics of germination of seed material was interested — whether there were changes of this indicator after deep freezing. The comparative analysis of germination of seeds in control, after fast freezing and freezing with a cryoprotector was carried out (Fig. 6).

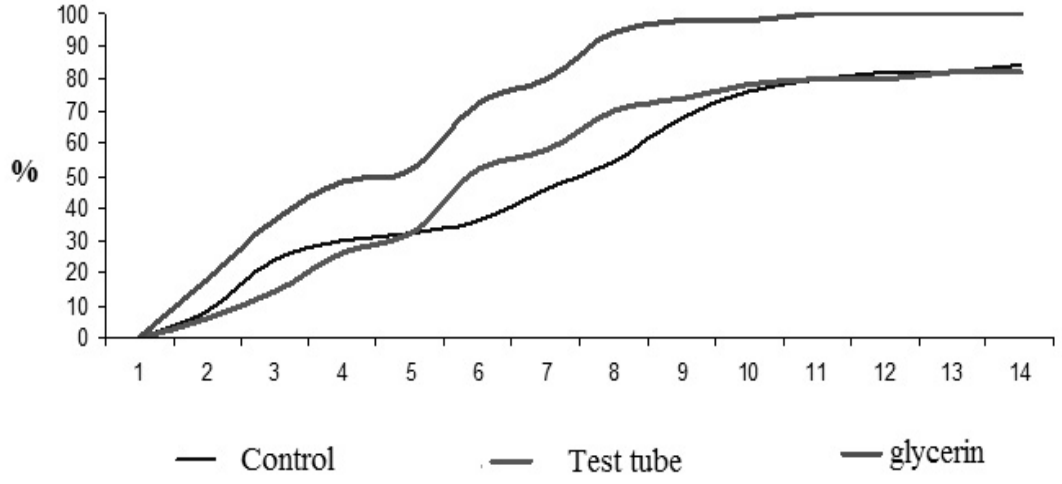


Figure 6. Comparison of dynamics of germination of seed material of *Serratula kirghisorum* in various options of a cryoconservation

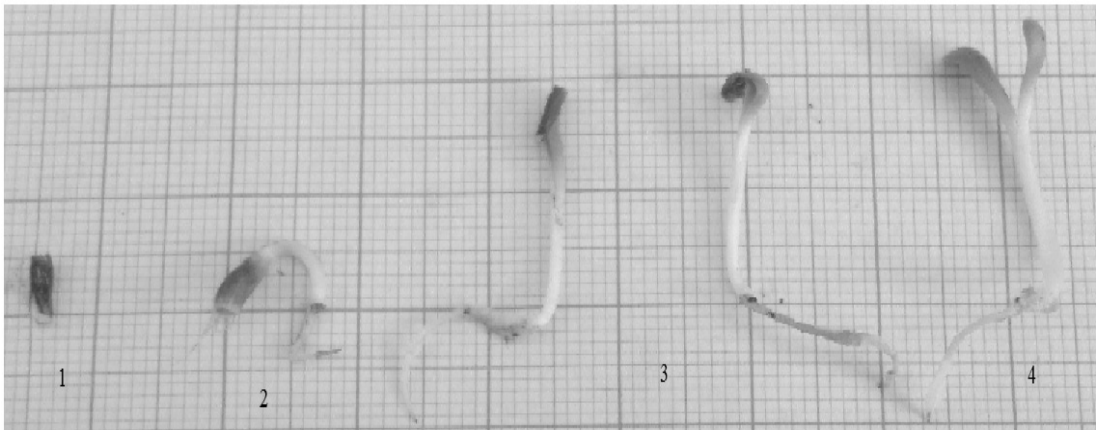
Seeds, which have been subjected to a deep freezing without cryoprotector, have shown preservation of initial viability; however there were less amicable germination of biological material. The seed material frozen with a cryoprotector has higher rates of viability, above than initial, and character of a curve hasn't changed, germination was amicable.

The biology of germination of the seed material of *Serratula kirghisorum* in normal conditions and after cryostorage has been studied. Beginning of shoots of seeds was observed for 3 days, for the 4th days on dried part of a seed there appeared the white germinal root 2,5 mm long and 1 mm wide, with well-expressed rootcap. The appearance of a hypocotyl with white color was observed for the 5th day, by this time length of the root made 8–9 mm, width was 1–1,5 mm. For the 7th day the hypocotyl of 14 mm long is slightly extended and took out cotyledonous leaves. For 10–11 days disclosure of cotyledonous leaves is observed, they were lanceolate form, green color, and smooth, smooth-edged with well-expressed central vein. By this time length of a root increased to 22–24 mm and to 1–1,5 mm wide, length of a hypocotyl of light green color made 15–16 mm, to 1 mm in the diameter. For the 13th day of germination the sprout had the following parameters: sprout length was 55 mm, length of a root (white color with a brown shade) was 28 mm, diameter of root was until 1 mm, diameter of a root neck was 2 mm, diameter of a hypocotyl was until 1,5–2 mm, length of cotyledonous leaves was 12 mm, width of a cotyledonous leaf was 5 mm, length of a scape of cotyledonous leaves was 4 mm (Fig. 7).

Essential distinctions in biology of seed germination after a cryopreservation were not revealed. The sprout passed all phases of development; the sizes of the main parts of sprout had not considerable differences. After freezing in liquid nitrogen passing by a sprout of phases of development happened slightly quicker, on average for one day, besides sprouts were more viable and strong. All results testified about safety of a cryopreservation for a seed germ, and allowed to use this method for preservation of seed material during creation of a collection of endemic plants' seeds.

In general low and ultralow temperatures influence viability on seed towards increase that is obviously connected with the fact that studied object belongs to orthodox seeds with organic type of rest, and also with sharply continental climate.

On the basis of the conducted experiments we can make the conclusion that during introduction into cryobank of genetic resources seeds of *Serratula kirghisorum* should be frozen with using of glycerin as a cryoprotector and to apply fast defrosting on a hot water bath.



1 — emergence of a germinal root; 2 — appearance and lengthening of a hypocotyls;
3 — carrying out outside of cotyledonous leaves; 4 — disclosure of cotyledonous leaves

Figure 7. Biology of germination of *Serratula kirghisorum*

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Serratula kirghisorum тұқымдық материалдарын мұздату арқылы сақтау

Мақалада криоконсервацияның әр түрлі әдістері арқылы қырғыз түймебасының тұқымдық материалдарының өнгіштігі мен тіршілік қабілеттілігі зерттелді, ыдыстардың әсерлері, мұздату және еріту әдістері, криопротекторларды пайдалану анықталды. Криопротектор ретінде глицеринді, пластикалық түтіктерді пайдалану кезінде ең жақсы көрсеткіштер алынды. Тұқымдық материалдарды ерітуді су буында (ваннасында) жылдам жүргізу қажет. Криодепонирлеудің әр түрлі әдістері арқылы тұқымдық материалдардың өніп-өсу биологиясы зерттелді. Криоконсервациядан кейін тұқымдардың өніп-өсу биологиясында айтарлықтай айырмашылықтар байқалмады. Өскін дамудың барлық сатысынан өтеді, өркеннің негізгі бөліктерінің өлшемдерінде айтарлықтай айырмашылықтар жоқ. Сұйық азотта мұздатудан кейін өскіннің даму сатылары орта есеппен бір тәулікте жылдамырақ өтеді, сонымен қатар өскіндер біршама өміршең және мықтырақ келеді. Осының барлығы тұқымның ұрығы үшін криоконсервацияның қауіпсіздігін дәлелдейді және сақтаудың осы әдісін тұқымдық материалдарды эндемик өсімдіктердің тұқымдарын жинақтау үшін пайдалануға мүмкіндік береді.

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Криосохранение семенного материала *Serratula kirghisorum*

Изучены всхожесть и жизнеспособность семенного материала серпухи киргизской при различных методах криоконсервации, определено влияние тары, метода замораживания и размораживания, применения криопротекторов. Наилучшие показатели, отмечено в статье, были получены при применении глицерина как криопротектора, пластиковых пробирок; оттаивание семенного материала необходимо было проводить быстро, с применением водяной бани. Изучена биология прорастания семенного материала при различных методах криодепонирования. Определено, что существенных различий в биологии прорастании семян после криоконсервации не обнаружено. Авторами подчеркнуто, что проросток проходит все фазы развития; размеры основных частей побега значительных отличий не имеют; после замораживания в жидком азоте прохождение проростком фаз развития происходит чуть быстрее, в среднем на одни сутки, кроме того, проростки более жизнеспособные и крепкие. Все это, определено в статье, свидетельствует о безопасности криоконсервации для зародыша семени и позволяет использовать данный метод сохранения семенного материала при создании коллекции семян эндемичных растений.

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