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Using of cryoprotectors for low-temperature storage of seed material of *Thymus rasitatus*

In article influence of various cryoprotectors on preserving viability of the seed material of *Thymus rasitatus* is studied and described. As a result of the conducted researches it is established that for *Thymus rasitatus* seeds the best storage conditions allowing to keep the highest rates of viability is use of a plastic container, a cryoprotector — 5% DMSO and slow defrosting at the room temperature. At the same time increase in level of energy of germination and viability by 15% and 10,7% in comparison with control is observed. After analyzing dynamics of seed germination of the researched species it was established that the seeds which were previously processed by glycerin and 5% DMSO with the subsequent cryopreservation sprout quicker and phases of development pass rather.

Keywords: Thymus rasitatus, cryoprotector, cryoconservation, DMSO, glycerin, germination, germinative energy, endemics.

Introduction. Degradation of wild conditions of the nature is resulted by reduction of number and destruction of populations of rare, endangered and endemic species. Loss of any species is irreplaceable loss in genetic and biological diversity of Kazakhstan flora. Studying of modern ways of preservation of the endemic plants which especially have economic and valuable value has important theoretical and practical value for preservation and restoration of a biodiversity, and also for ensuring requirements of the pharmaceutical and medical industry of Kazakhstan.

The important direction of researches can be considered creation of seed banks of endemics, because they have a limited area of distribution and meet only in a certain territory. It is well-known that storage of seeds at positive temperatures leads to decrease in their viability because of physiological processes, accumulation of mutations and damage of a germ. Now the cryopreservation is considered a new method of storage of plant material that allows keeping viability and physiological full value of seed.

Object of investigation is seed material of *Thymus rasitatus* Klok. from family *Lamiaceae*, collected in the mountains Bektauata at the beginning of August, 2015. *Thymus rasitatus* is a semi-bushes, 3-8 cm high, with the rising and highly woody branchy trunks and the generative ascending shoots on apexes and side branches, a crown violet, an inflorescence capitated, leaves petiole simple, oblong and elliptic. This species is the endemic of Kazakhstan growing on low hills, cracks of rocks at the exits of granites, petrous taluses. It meets in the mountains Karkaraly, Kent, Niyaz, Bektauata, Ortau, Kyzyltau, Chingistau [1]. It is a valuable officinal and essential oil plant [2].

Medical properties of *Thymus rasitatus* is caused by presence essential oil, polyphenol compounds, tannins, bitterness, gums, organic acids, pitches, fat oils, mineral salts and vitamins [3]. *Thymus rasitatus* has antimicrobial, anti-inflammatory, anti-oxidant and spasmolytic activity. An elevated part of species of a thyme is used in official and traditional medicine as the liquid extract and drug of Pertussinum possessing expectorant and anti-microbe action [4].

Methodology

Seeds were exposed to fast freezing in liquid nitrogen (-196 °C). Freezing of material was carried out in plastic test tubes (Nunc brand) with use of two types of cryoprotectors - glycerin and DMSO of different concentration (1%, 3%, 5%). Two modes of defrosting were applied: fast – on a water bath at a temperature of +80 °C, slow — in case of indoor temperature (+22 °C) [5–7].

The research of viability and energy of germination of seeds was conducted by methodical instructions of M.S. Zorina, S.P. Kabanov and M.V. Maltseva [8, 9].

In vitro seeds were couched in the Petri dishes in 4-fold frequency on 2-layered filter paper moistened with the distilled water. Disinfection of seeds was carried out by means of 0,5% of $KMnO_4$ solution with the subsequent laundering in the distilled water. Sprouting of seed material was realized in the climatic camera at a temperature +24 ^oC and a constant lighting.

Statistical processing of results was carried by N.L. Udolskaya's technique [10].

Results and their discussion

In the process of carrying out researches control viability of seeds of *Thymus rasitatus* with after 9 month period of storage was 29,3±0,9%, and energy of germination was 23±1,0%, fresh-gathered seeds haven't sprouted. According to literary data at a cryopreservation render cryo protector substances since protect the frozen material from the damaging action of extremely low temperatures on extent of preservation of viability of seeds, reduce extent of formation of crystals of ice. By results of researches it has been established that seeds of *Thymus rasitatus* with full physiological ripening sufficiently keep viability after freezing in liquid nitrogen. Action of the applied cryoprotectors consists in decrease in amount of free intracellular water and increase in viscosity of internal solutions.

Earlier experiments by an assessment of viability and energy of germination of seeds after a cryopreservation in liquid nitrogen have been conducted. It was revealed that the seeds frozen in plastic test tubes had viability from 20,7% to 24,7% depending on defrosting conditions. In comparison with initial indicators the percent of viability of seeds is 4,6% lower, but, nevertheless, seeds have kept viability [11].

Before freezing in liquid nitrogen seeds immersed in glycerin and DMSO of various concentration, plunged in conditions into Dyuar's vessel.

At a cryopreservation with use of cryo protective substances, such as glycerin and 5% — DMSO solution indicators of germination have improved, in comparison with control values. The best viability of seed material was shown by the seeds frozen in 5% DMSO solution (43,7±0,8%) and refrozen at the room temperature (Table 1).

Table 1

Indicators of seed germination of *Thymus rasitatus* after cryo conservation with using of cryo protectors

Cryo protector	Energy of germination,%		Seed germination,%	
	Slow refrozen	Fast refrozen	Slow refrozen	Fast refrozen
Without cryo protector - control	8,3±0,9	20,7±1,1	13,4±0,8	24,7±0,9
Glycerin	38±0,5	13,9±1,0	40±0,7	15,8±0,6
1% DMSO	20±0,7	-	24±1,0	-
3% DMSO	24±0,9	-	26±0,9	-
5% DMSO	39,5±1,0	-	43,7±0,8	-

The conducted researches have proved big efficiency of the cryo protective properties of DMSO in comparison with glycerin. Use of DMSO at a cryopreservation ensures higher safety of viability of cells of seed. The best indicators of viability after cryogenic storage have shown the seeds placed in 5% DMSO solution or glycerin frozen in plastic tubes and which is slowly refrozen at the room temperature — 43,7±0,8% and 40±0,7% respectively (Fig. 1).

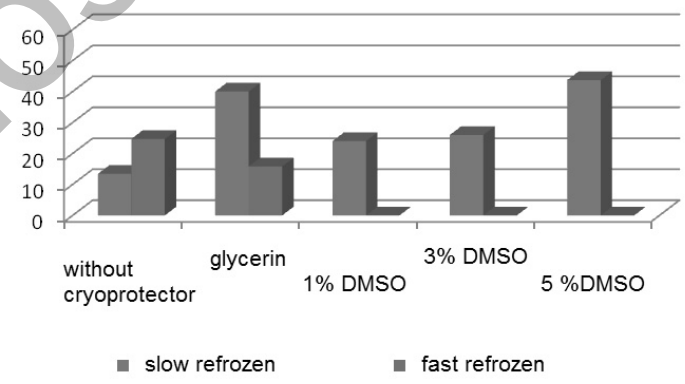

Figure 1. Seed germonation of seed materials of *Thymus rasitatus* after influence of extreme low temperature

Viability of seeds of *Thymus rasitatus* at use glycerin has increased to 40±0,7% that above control values for 10,7%. At approbation of DMSO as cryprotector it has been established that the best concentration

are 5% solution — 43,7±0,8%. When using 1% DMSO solution viability has made 24±1,0% that is 5,3% lower than control values, and when freezing in 3% — 26±0,9%, below indicators of control group for 3,3%.

When comparing the indicators of viability observed at influence of glycerin and 5% DMSO, the best result shows DMSO.

Energy of germination has increased at the seeds processed by glycerin to 38±0,5% in comparison by simple freezing. When using 1% DMSO solution energy of germination has decreased to 20±0,7%, 3% and 5% DMSO has increased to 24±0,9% and 39,5±1,0% respectively (Fig. 2).

The slow way of refrozen for seeds of *Thymus rasitatus* is the best option at a cryopreservation with use of cryoprotectors, viability at this way has made from 24±1,0% to 43,7±0,8% that above control values on average for 16,5%. Fast refrozen leads to lower preservation of viability of seeds of the studied species — from 0 to 15,8±0,6%, in comparison with defrosting of seeds at the room temperature that is much lower than control data. Energy of germination in comparison with reference values has increased on average na19,5%, viability for 16,5%.

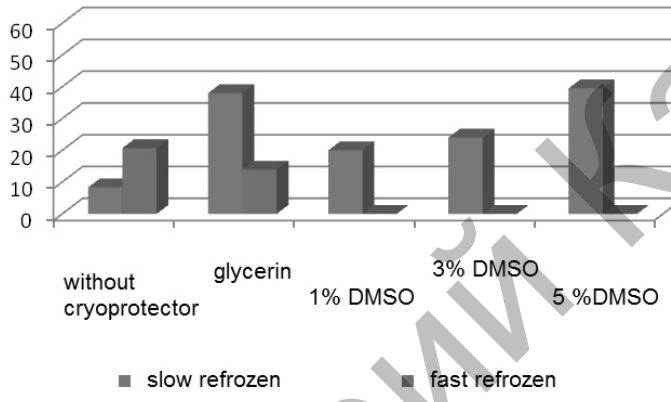

Figure 2. Emergy of germination of seed materials of *Thymus rasitatus* after action of extreme low temperature

After analyzing dynamics of germination of seed material, it has been established that the seeds which are previously processed by 5% DMSO have shown the best viability in comparison with other options of experience and control which has made 12,5% in the first day of germination and for the 10th day — 39,5%. And the seeds which have undergone processing by 1% and 3% DMSO have begun to sprout later for 1–2 days (Fig. 3, Table 2).

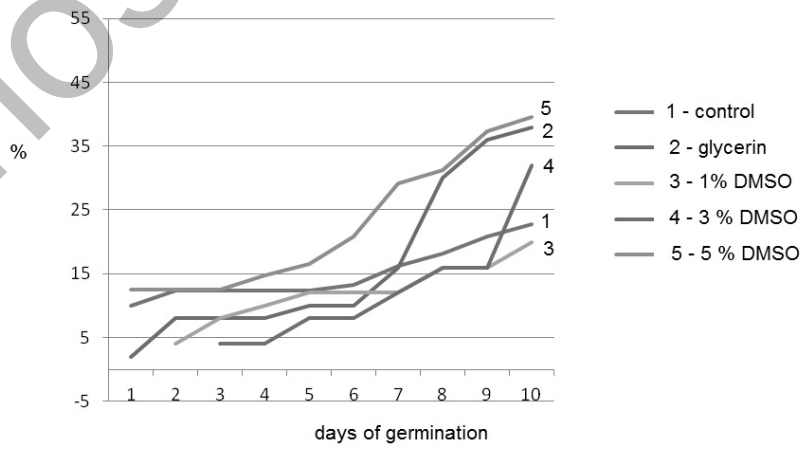

Figure 3. Dymnamic of germination of seeds of *Thymus rasitatus*, which are previously processed cryo protectors

Table 2

Dynamic of germination of seeds of *Thymus rasitatus* processed by cryo protector and fast frozen

Type of experiment	Day of germination / energy of germonation,%									
	1	2	3	4	5	6	7	8	9	10
Without cryo protector - control	10	12,4	12,4	12,4	12,4	13,3	16,2	18,1	20,9	22,8
Glycerin	2	8	8	8	10	10	16	30	36	38
1% DMSO	0	4	8	10	12	12	12	16	16	20
3% DMSO	0	0	4	4	8	8	12	16	16	32
5% DMSO	12,5	12,5	12,5	14,7	16,6	20,8	29,1	31,2	37,4	39,5

In general after analyzing percent of viability it has been established that the seeds shipped in glycerin and 5% DMSO with the subsequent cryopreservation sprout quicker and phases of development pass rather.

Conclusion. Thus, it is established that for *Thymus rasitatus* seeds the best storage conditions allowing keeping the highest rates of viability is use of a plastic container, a cryoprotector — 5% DMSO and slow defrosting at the room temperature. At the same time increase in level of energy of germination and viability by 15% and 10,7% in comparison with control is observed. So, the offered ways of low-temperature storage will allow to deposit *Thymus rasitatus* seeds unlimited time without harm for a germ of a seed and loss of indicators of viability and energy of germination that, in turn, will give the chance to enter this species of an endemic plant into a collection of a genetic variety.

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Thymus rasitatus тұқымдық материалының төмен температурада сақтау кезінде криопротекторларды қолдану

Мақалада *Thymus rasitatus* тұқымдық материалының өнімділігін сақтауға әр түрлі криопротекторлардың әсері зерттелген. Нәтижесінде *Thymus rasitatus* тұқымы өнімділігін жоғары көрсеткіштерін сақтауға мүмкіндік беретін ең қолайлы жағдайы пластикалық ыдыстарды, 5%-дық ДМСО криопротекторды және бөлме температурасында баяу жібітуді пайдалану болып табылады. Сонымен қатар, бақылаумен салыстырғанда, тұқымдардың өну қарқындылығы мен өнімділігінің өсуі 15 және 10,7 %-ға артқаны байқалады. Зерттелген тұқым түрінің өсу динамикасын талдау барысында алдын ала глициринмен және 5 %-дық ДМСО криоконсервациядан кейін жылдам өнеді және даму фазалары жедел жүретіндігі анықталды.

Кілт сөздер: Thymus rasitatus, криопротекторлар, криоконсервация, ДМСО, глицерин, өнімділік, өну қарқындылығы, эндемик.

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Использование криопротекторов при низкотемпературном хранении семенного материала *Thymus rasitatus*

В статье изучено и описано влияние различных криопротекторов на сохранение жизнеспособности семенного материала *Thymus rasitatus*. В результате проведенных исследований установлено, что для семян *Thymus rasitatus* наилучшие условия, позволяющие сохранить наиболее высокие показатели всхожести, — использование пластиковой тары, криопротектора — 5%-ного ДМСО и медленного размораживания при комнатной температуре. При этом наблюдается увеличение уровня энергии прорастания и всхожести на 15% и 10,7% по сравнению с контролем. Проанализирована динамика прорастания семян исследуемого вида. Установлено, что семена, предварительно обработанные глицерином и 5%-ным ДМСО, с последующей криоконсервацией, прорастают быстрее, и фазы развития проходят скорее.

Ключевые слова: Thymus rasitatus, криопротекторы, криоконсервация, ДМСО, глицерин, всхожесть, энергия прорастания, эндемик.

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