14. Материалы сайта: www.nkj.ru
15. Материалы сайта: http://www.cseemc.kz/uploads/stat2.pdf#page=4&zoom=auto,-107,144

D.Yu.Sirman[1], S.U.Tleukenova[1], L.B.Arystan[1]

THE EFFECT OF STEP WISE FREEZING OF SEEDS OF SOME CONIFEROUS SPECIES DEPENDING FROM RESISTANCE TO CRYOFREEZING AND ASSESSMENT LABORATORY GERMINATION

Karaganda State University named after academician Ye.A. Buketov, Kazakhstan

Cryopreservation is preservation at extremely low temperatures (using liquid nitrogen) of living biological objects with the possibility of restoring their physiological functions after defrosting [1]. In technical terms, this method is easier, cheaper, more reliable, and environmentally calm than usual refrigeration. [2]

Deep freezing can last from a few minutes to a number of months and years. Evaluation of the success of cryopreservation is usually determined by laboratory seed germination [2]. Today, an important indicator for cooling and freezing is the speed of the process, as this directly affects the experiences. Neither mechanical freezers, nor traditional shockers provide a high cooling rate. For comparison, the time of freezing in a mechanical freezer or in a Shocker is several hours, and for freezing by cryogenic method it takes only a few minutes. Also, with ultra-fast freezing, it is possible to avoid internal damage and cell damage due to the fact that the ice crystals formed have smaller dimensions, due to the increase in the freezing rate [3].

A deep species-specific reaction to freezing of plant seeds is differing from types of natural populations or years of collection [7].

In 1989, studies By the national laboratory for seed storage of the United States found that low positive temperatures can guarantee the viability of the seeds of most cultivated species at the initial level only for 5-10 years of storage, and shallow freezing (-18 °C and below) – up to 10-20 years. In Portugal, summing up the storage of seeds of rare plants for 2 and 4 years showed that half of the studied species (14 of 28) had a shallow freezing (-17°C), which led to a sharp drop in viability even compared to storage in room conditions. Therefore, the deep freezing regime (cryopreservation) was recommended for the most valuable genetic plant resources [2].

The accumulated results show that for the seeds of the majority cultivated species such type of storage does not lead to its reduction, but the aftereffects have not been studied enough [2]. There is evidence of a positive effect of deep freezing on seed germination – in particular, characterized by hardness [8, 12], as well as having a physical type of rest [11]. The study of the growth dynamics of seedlings

Репозиторий КарГУ

grown from Sophora flavescens's seeds stored in liquid nitrogen for 1 month showed that cryopreservation does not lead to the appearance of ugly specimens [4].

The possibility of long-term preservation of seeds and their successful cryopreservation is associated with their dehydration at maturity [1].

The study of metabolism in frozen tissue cultures, plant cells and microorganisms allowed establishing that as a result of shallow freezing (0 °C), the exchange processes continue, the activation of lipid peroxidation occurs, and as the temperature drops to -25 °C, these processes are even more intensified. In the membranes of plant cells, even at -60 °C, dynamic changes occur, and at -130 °C, crystallization and recrystallization phenomena are likely, excluding the possibility of long-term storage of frozen material [11] for some crops, the threshold of seed moisture was revealed, above which successful cryopreservation requires the use of cryo protectors [8] all cell metabolism stops only in liquid nitrogen at -196 °C. [10, 6, 5]

Methodology. Experiments to study stability of seeds to cryogenic freezing performed at the laboratory «Biotechnology and molecular genetics» of KSU.

Researching objects were seeds of 2 plant species plants of *Pinaceae* family: *Picea asperata* and *Picea pungens*. These seeds are collected on September and saved on standard conditions in the paper package (temperature +15°C, humid 77%).

Before the researching are defined mass and humiliated for 1000 seeds (table 1). Weighing seeds conducted at the analytical scales NPV 220 in three-fold repetition with calculation of average value of weight of seeds.

Table 1 – Mass and humidity of studied seed

Name of species	Mass of 1000 seed, g	Humidity, %
Picea pungens (Engelm)	3,66±0,03	2.3%
Picea asperata (Masters)	5,34±0,19	4%

Cryogenic freezing of seeds conducted in three stages by various time of freezing (table 2): 1 stage–freezing in refrigerator +4°C; 2 stage – freezinginfreezer-8°C; 3 stage –freezing in liquid nitrogen -196°C.

Table 2 - Variability of time intervals of the various stages in the freezing of seeds

№ variation	1 stage	2 stage	3 stage
1 variation	1 hour	1 hour	168 hour
2 variation	12 hour	12 hour	168 hour
3 variation	24 hour	24 hour	168 hour
4 variation	72 hour	72 hour	168 hour
5 variation	168 hour	168 hour	168 hour

After freezing seeds subjected to defrosting by reversing technology. To their defrosting during one hour in every stage. After defrosing seeds desinfescted with

Репозиторий КарГУ

help of 0,5% solution of $KMnO_4$, then washed with distil water. Seed germination is determined in crystallizer in climatic camera "Binder" KBW 240 with the artificial lighting (24 h) and temperature of +23 °C. As a control is planted the seeds of this species previously expose their stratification for during of 3 month. Stratification spent cotton fabric, placing to wet sand. Container is placed to refrigerator camera with temperature +4.

Statical processing results led by the method N.L. Udolskaia [9]. Viability of seeds are estimated by laboratory germination. Energy of germination defined on the 15 day, seed germination is defined on the 20 day after planting.

Medium seeding peace calculated by a formula:

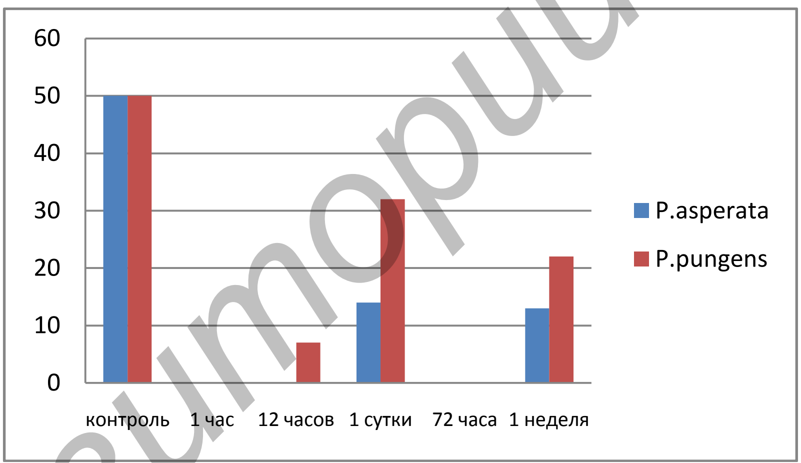
$P = (a_1t_1 + a_2t_2 + \ldots a_n t_n) / (a_1 + a_2 + \ldots a_n)$,

Where - $t_1, t_1, \ldots, t_n$ – days where beginning germinated seeds;

$a_1 + a_2 + \ldots a_n$ – amount of seeds germinated on this days.

Results and discussion. Previously analysis of experiment showed that various impact stage freezing seeds of two species in Masters and Engelm.

Analysis of results showed, that the best seed germination observed for both species in third variation of freezing. So, for Engelm seed germination was 32% and for Masters - 14% (figure 1).



Picture 1 - Germination relative indicator of the seeds in different variation cryo freezing

Relative, highly indications of energy of germination are received for Engelm in third and fifth variations - 24% and 22%. For Masters significant indications of germination energy are noted in third variation - 14% (figure 2).

Репозиторий КарГУ

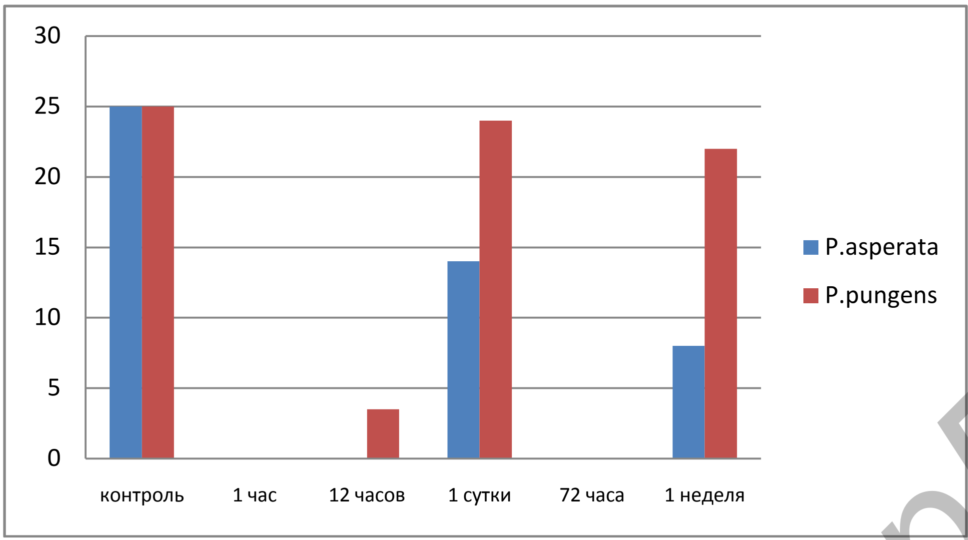


Figure 2 - Relative indicators of energy germination of seeds in different variation of cryo freezing

Comparing of results with control data established reducing seed germination and germination energy. For Engelm seed germination reduced to 18% (taking into account the variation of the greatest germination); for Masters seed germination reduced to 36% (figure 3).

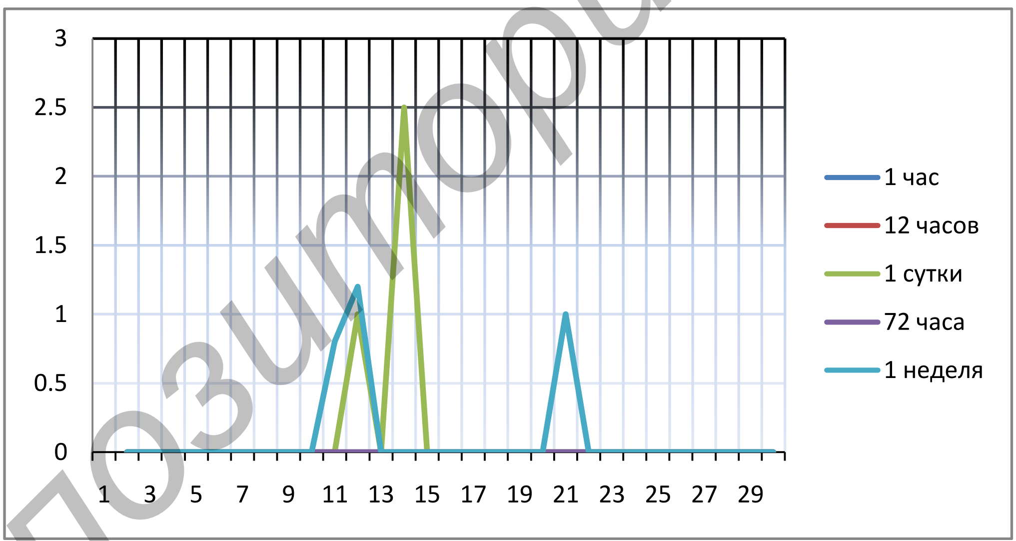


Figure 3 - Changing of dynamics seed germination of Masters

Analysis of changing dynamics of seed germination showed that different variation cryo freezing provided unequal effect. So, for *Picea asperata* is fixed two peaks of active seed germination.

Difference between the peaks by variants of experiments was from 2 till 9 days. So, in 3th variant rest period after defrosting was 13,4 days; in 5th variants - 14,7 days. In control variant this result was 20,8 days.

Analysis changing dynamics of seed germination of Engelm exposed that quantity periods with intensive germination varied depending on the type of freezing.

Репозиторий КарГУ

So, in 2nd variation we can see four peaks of seed germination, third peak from their show more mass germination of seed (figure 4).

On third variation first peak more significantly relative to next two peaks. On fifth variation in difference from two previous variations observed only two peaks, which from their second was more highly. The difference between experimental data and control was 2-5 days.

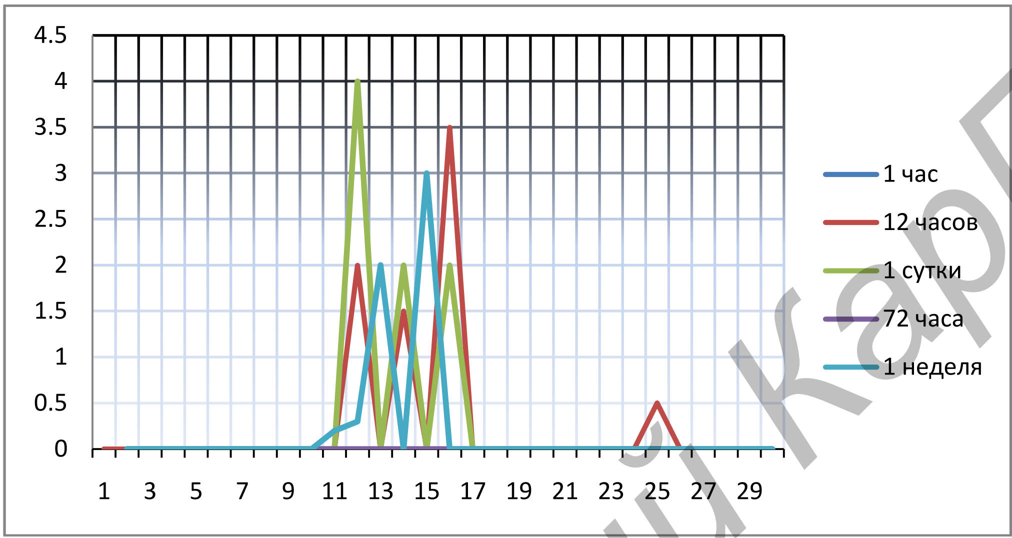


Figure 4 – Changing of seed germination's dynamics of Engelm

Conclusion. Stepwise freezing of seeds reduced germination and energy of germination for both species. Comparing experimental data is shown that results of seed germination after stepwise freezing exceeded control results on 50%.

Optimal results were on sequential freezing of seeds with daily exposure on each stage. For both researching species was regularity between exposure freezing seeds and their germination. Cryo freezing of seeds multidirectional influences rest energy of seeds.

Reference

1. Wikipedia – free encyclopedia. Cryopreservation // Wikipedia – free encyclopedia / Wikipedia – electronic data – Access mode: http://ru.wikipedia.org/wiki/ – Title of article – language. Russian. (Date of application: 20.10.2018).

2. Tihonova V.L. Long term storage of seeds // Physiology of plants. – 1999. – T. 46. - № 3. – P. 467-476.

3. Rashitova S. Cryogenic cooling and freezing from LINDE GAZ RUS // All about meat. – 2014. - № 4. – P. 41.

4. Molodkin V.U. Value wet of seeds some cereals and grain legumes during cryopreservation in liquid nitrogen // Bulletin. – 1986. – № 165. – P. 22-24.

Репозиторий КарГУ

5. Tihonova V.L. Influence of low and ultra low temperature to storage on laboratory germination seeds agrestic herbaceous plants 1. Seeds without period of rest // Cryobiology . – 1990. – № 4. – P. 23-28.

6. Gresshoff P., Gartner P. Cryopreservation of Arabidopsis thaliana and other seeds by storage in liquid nitrogen // Arabidopsis Inform. Serv. - 1977. - V. 14. - P. 12.

7. Voronkova N.M. Morphobiological characteristic and reaction to cryoconservation seeds some species of flora Kuril Islands // Floral resources. – 2000. – T. 36 - № 4 – P.40-47.

8. Smirnov I.A. Storage genetical recources representatives family Fabaceae in banks // Problems of botany on the abroad. XX-XXI centuries: thesis of report submitted to congress. – St. Petersburg, 1998. - Vol. 2. - P. 324.

9. Udolskaia N.L. Introduction to Biometrics. – Almaty: Science, 1976. – 84 p.

10. Sakai A., Noshiro M. Some factors contributing to the survival of crop seeds cooled to the temperature of liquid nitrogen // Crop Genetic Resources for Today and Tomorrow. – 1975. – P. 317.

11. Butenko O.U., Bondarenko A.S., Pelevina N.N. Influence of modes cryofreezing and thawing to germination of seeds pines and firs // Proceedings of Sank-Petersburg scientific research institute of forestry. – 2014. - № 1. - P. 38-46.

С.У. Тлеукенова, А.К. Рамазанов, Н.Е. Рахимгерей, С.М. Әбдіғалым,
А.И. Ахметжанова, К.Б. Бекишев

DATURA STRAMONIUM ТҰҚЫМДЫҚ МАТЕРИАЛЫН КРИОСАҚТАУ КЕЗІНДЕ КРИОПРОТЕКТОРЛАРДЫ ҚОЛДАНУ

Карагандинский государственный университет им. Е.А.Букетова, Казахстан

Қазақстан территориясында фармакопейлік тізімнен 45 жабайы түрі кездеседі. Қазақстанда дәрілік өсімдіктердің ең көп тараған түрі : жалаңаш мия тамыры, сафлора тәрізді маралтамыр, кәдімгі жұпаргүл, долана түрлері, кәдімгі мыңжапырақ, түймебас және тасшөп түрлері, дәрілік қандышөп, кәдімгі гармала және т.б. [1-2].

Бұл өсімдік түрлері әртүрлі шикізат дайындаушы және халықпен үлкен көлемде жинақталады. Оңай қол жетімді, жақсы танымал орындарды жүзеге асырылатын бақыланбаған шикізат дайындаушылары, тұрақты пайдаланылатын популяцияда құлдырауға ұшыратады.

Мекен ету ортасының табиғи жағдайдағы деградациясы нәтижесінде өсімдіктердің сирек кездесетін және эндемик түрлері саны жағынан қысқаруға ұшырайды. Кез келген түрін жоғалту, Қазақстанның флорасының генетикалық және биологиялық флорасының әртүрлілігінің шығынына әкеліп соғады. Экономикалық тұрғыдан құнды болып келетін эндемдік түрлердің сақтау тәсілдерін зерттеу барысында, қазіргі таңда теориялық және практикалық

Репозиторий КарГУ