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Effects of carbohydrate content in seeds on the germination and viability after cryopreservation

This article is devoted to the study of the influence of cryopreservation on the preservation of sowing qualities of seeds of representatives of the genus *Picea*: *P. asperata* (Rough spruce) and *P. pungens* (Spiny spruce). As an alternative shock method of cryopreservation, the method of step-by-step 3-step temperature reduction was used 1 stage +4 °C, 2 stage –18 °C, 3 stage –196 °C liquid nitrogen. Additionally, the effect of 4 time intervals (1 hour, 24 hours, 72 hours and 168 hours) of step freezing on seed quality was investigated. To explain the results by photometry, the total carbohydrate content of the seeds is determined before the 3rd freezing step (cryo interaction). The analysis of the results of germination showed that the seeds of the studied species respond in different directions to the step cryo freezing. So, seeds of *P. asperata* germination in all tree experimental variants are higher on 4–32 % than in the control variant; and the maximum results are revealed in the variant with time intervals of 168 hours. For *P. pungens* seeds, the germination rates in experimental variants were on 18–22 % lower than in the control; and the largest germination rate of seeds among experimental variations are revealed in variant with interval 24 hours. The determination of the total carbohydrate content of the tested seeds at different cryogenic freezing intervals found that this indicator varied significantly depending on the time interval. The lowest values of total carbohydrates are found in variation with a time interval — 24 hours. The maximum total carbohydrate content is determined in a variation with a time interval — 1 hour. Correlation analysis between seed germination indices and total carbohydrate content established a high relationship between these indices. So, for *P. asperata*, the value of the Pearson coefficient was 0.86, and for *P. pungens* seeds — 0.91.

Keywords: cryopreservation, seeds, nitrogen, germination, viability, hydrocarbons, *Picea pungens, Picea asperata.*

Introduction

Cryopreservation is an optimal method of unlimited long-term storage of the plant gene pool by freezing plant material at an extra low temperature (–196 ºC) [1]. This method allows preserving the cells and protoplasts of various plants, including plants that cannot withstand dehydration, such as meristems, shoots, zygotic and somatic cells, pollen and some types of seeds [2].

The long-term storage of seeds depends on a number of internal and external factors: species characteristics, the degree of their ripening, the density of the seed bark, the composition of the main auxiliary substances, as well as humidity during the storage, temperature and aeration. The experience of T.P. Orekhova showed that the degree of productivity of seed material after cryogenic freezing is influenced by the level of humidity of seeds and the content of nutrients in them [3].

The most widespread is the low-temperature storage of plant materials under *in vitro* conditions. Low-temperature freezing is also important because with a decrease in temperature, the coefficient of volume expansion of ice decreases [4]. The studies are carried out on woody plants by the storage of cultured material in liquid nitrogen at a temperature –196 ºC [5].

It is known that cells of vegetating plants contain endogenous osmotic active substances such as mono- and disaccharides (glucose, fructose, sucrose), organic acids, amino acids and other compounds [6]. Their significance and characteristic feature is influence the viability of plant cells and tissues to varying degrees after cryopreservation processes of plants, followed by their storage at ultra-low temperatures in liquid nitrogen vapor (–183–185 ºC) [7].

To successfully perform cryopreservation of various bio-objects, it is necessary to concentrate biopolymer solutions in plant cells [8]. This method protects them from the lethal action of intracellular ice crystals. In addition, partial dehydration and concentration of intracellular solution prevents the occurrence of pressure destroying cells from the inside due to ice expansion. Therefore, the seed material for freezing and further cryopreservation is taken in a state of deep rest [9]; the seeds must be dried to remove free water.

The accumulation of sucrose in plant tissues positively affects the cryopreservation of shoots of fruit and berry crops. Hydrolysis of sucrose until mono-sugars negatively affects on the survival; and organic acids take an intermediate effect on the cryopreservation process. High accumulation of amino acid proline in plant cells under stress conditions can significantly increase the ability to detoxify and reduce oxidative damage.

Seeds of such arid plants as cacti, after drying, usually retain their germination [10]; their samples are able to storage at cryogenic temperatures for a long time without significantly viability reducing [11–13].

The purpose of the present study is to determine the effect of carbohydrates in spruce seeds on the survival of plant material.

Materials and Methodology

Experiments are carried out in the laboratory of biotechnology and molecular Genetics of the Karagandy University of the name of academician E.A. Buketov in 2018–2019. The objects of the study are seeds of species of genus *Picea* A. Dietr. (Spruce): *P. asperata* (Rough spruce) and *P. pungens* Engelm. (Spiny spruce). Seeds are obtained in October, 2018, according to the Index Semenium exchange between the Moscow Nursery and Karagandy University of the name of academician E.A. Buketov. Seed materials of *P. asperata* and *P. pungens*e before experiments are stored at temperature +15 ºC for 1 month.

The cryogenic freezing of seeds is performed in three stages with different freezing intervals (Table 1):

– 1 stage — freezing in refrigerator +4 ºC;

– 2 stage — freezing in the freezer –18 ºC;

– 3 stage — freezing in liquid nitrogen –196 ºC.

Table 1

Variability of time intervals of different stages of seed freezing (in hours)

Number of variant	1 stage	2 stage	3 stage
1 variant	1	1	168
2 variant	24	24	168
3 variant	72	72	168
4 variant	168	168	168

Defrosting of seeds is carried out in a refrigerating chamber at a temperature of +4 ºC for 1 hour. Before sowing, the seeds are disinfected by 0.5 % $KMnO_4$ and chlorine solution, each per 7 minutes. After sterilization, the seeds are washed three times in sterile distilled water with further drying. For seed germination, a bath type crystallizer is used; germination is carried out in the climate chamber «Binder» KBW 240 with artificial lighting of 4000 lux, during 24 hours and at the temperature +23 ºC. Seeds are planted on filter paper in a crystallizer — by 25 pieces in 4 repetitions. Seeds of spurs with stratification at +4 ºC for 3 months are used as controls.

In experiments are determined the following parameters: energy of germination, seed germination, seed rest period, carbohydrate content and loss of seed mass [14]. Energy of germination is calculated on 15^{th} day. Statistic processing is conducted by N.L. Udolsky [15].

Total carbohydrate content is determined on three variations of seeds by 50 pieces by photometric analysis techniques [16, 17]. The determination is carried out on Unico 1201 photo-spectrometer at a wavelength 320 nm. For calculation the total carbohydrate content, a calibration scale is built for the standard glucose solution in the range from 5 % to 55 % at 11 points, with a step 5 %.

Correlation analysis between total carbohydrate content values in seeds and germination indices of seeds is performed by Pearson coefficient.

Results and Discussion

Mass and humidity of 1000 pieces of seeds are determined on the analytical scales of NPV 220 in triplicate (Table 2).

Table 2

Mass and humidity of seeds of *P. asperata* and *P. pungens*

Species	Mass of 1000 seeds, g	Humidity, %
P. asperata	5.34 ± 0.19	4.0
P. pungens	3.66 ± 0.03	2.3

The results of cryogenic freezing of seeds showed that *P. pungens* seeds were more resistant to exposure extra low temperatures compared to *P. asperata* seeds. So, for *P. asperata*, the best result of seed germination is observed in the 4th variant — 22 %, for *P. pungens* the maximum germination is noted in the 2nd variant — 32 %.

In experiment the energy of germination of *P. asperata* was on 16 % higher than in control variant; for *P. pungens* — on 18 % lower than in control (Fig. 1, 2).

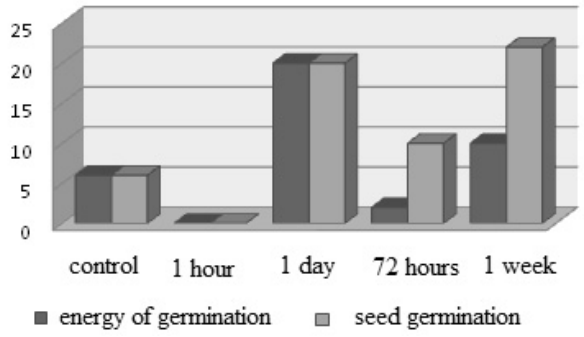

Figure 1. Seed germination and energy of germination of *P. asperata*

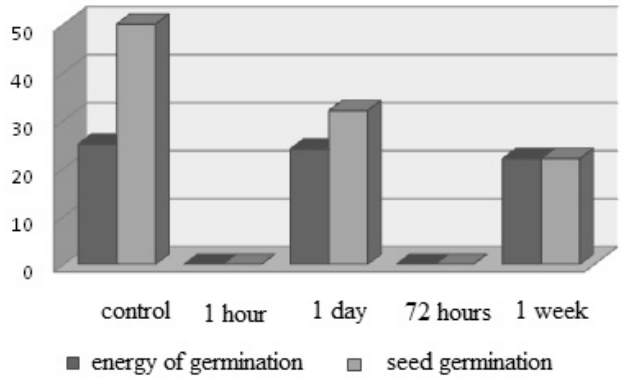

Figure 2. Seed germination and energy of germination of *P. pungens*

The smallest germination of *P. asperata* seeds is recorded in the 3rd variant (10 %); for *P. pungens* — in the 4th variant (22 %). Thus, *P. asperata* had the lowest energy of germination on 4 % higher than the control values. *P. pungens*'s was on 28 % lower than in control data.

The best indicators of energy of germination are noted in the 2nd variant of cryogenic freezing. So, for *P. asperata* the energy of germination was 20 %, for *P. pungens* — 24 %.

The results of the resting energy index after the step-by-step cryogenic freezing of the seeds revealed a different seed response according to the experience options. For *P. asperata* seeds the maximum resting en-

ergy indicator among experiment is recorded in the 3rd variant with an indicator 23.6 days; for *P. pungens*, the minimum value is noted in the 4th variant and amounted to 13.9 days. The minimum value of seed resting energy in the experiment is observed in the 2nd variation with 14 days for *P. asperata* and 13.5 days for *P. ungens* (Table 3).

Table 3

Parameters of the resting energy index of *P. asperata* and *P. pungens* after cryopreservation

Variants	The resting energy index, days	
	P. asperata	*P. pungens*
Control	6	13.3
1st variant	0	0
2nd variant	14	13.5
3rd variant	23.6	0
4th variant	17.6	13.9

The largest decrease in the mass of *P. asperata* seeds is observed in the 1st variant of the study, which was 3 % (Fig. 3).

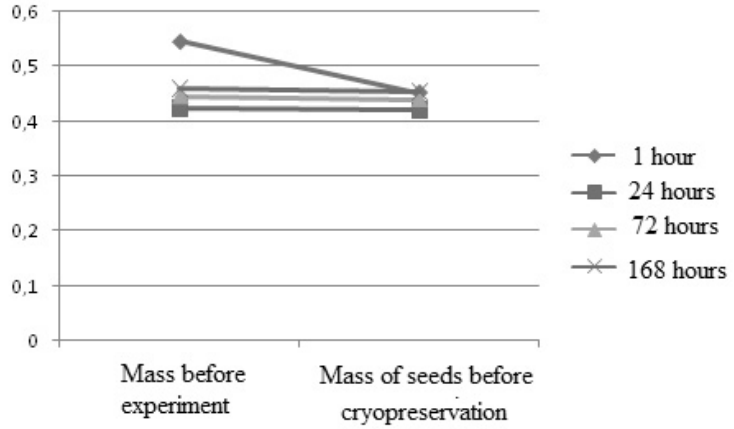

Figure 3. Change in mass of *P. asperata* seeds before cryopreservation

For *P. pungens* seeds, the most significant weight decrease was observed in the 3rd variant, where the seed weight degreasing was 1.68 % (Fig. 4).

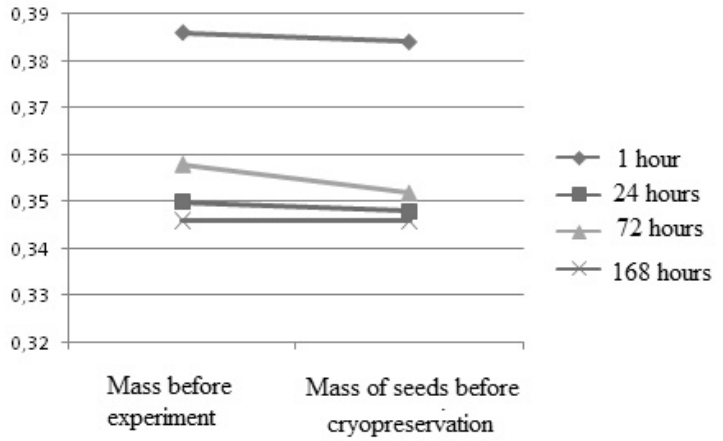

Figure 4. Change in mass of *P. pungens* seeds before cryopreservation

Photometric analysis of the content of carbohydrates showed that this parameter did not have any dependence on the duration of the cryo freezing stages. So, in *P. asperata* seeds, the lowest carbohydrate con-

tent is recorded in the 2nd variant (21 %). The maximum carbohydrate content is recorded in the 1st variation (52 %).

The difference between the maximum and minimum carbohydrate content in *P. asperata* seeds was 31 %. In *P. pungens* seeds the minimum carbohydrate level is noted in the 2nd variant (21 %) (Table 4).

Table 4

Relative total carbohydrate content at different stages of freezing (in %)

Species	168 hours	72 hours	24 hours	1 hour
P. pungens	40.0	***	21.0	59.0
P. asperata	24.5	42.5	22.0	52.0

Note. *** — the total carbohydrate content is outside the defined scale.

The correlation coefficient between carbohydrate content and germination for *P. asperata* seeds was 0.86; for *P. pungens* — 0.91, which indicates the presence of a high association between these indicators.

The results of the studies showed a significant difference in the reaction of seeds to cryogenic storage. In *P. asperata* seeds, in 3 variants of the experience, germination was higher than in the control, and amounted to 4 to 32 %. In *P. pungens* seeds in the experiment the germination was on 18–22 % lower than for control data. An important indicator is the duration of each of the freezing steps. The greatest indicators of germination of seeds were experience variants with time intervals of 24 and 168 hours. After one hourly interval of phased freezing, neither species seedlings appeared. Similar results are obtained in the experiments of G.V. Verzhuk [18]. The physical effect is due to large intracellular ice crystals, which form during rapid cooling and cause mechanical damage [19].

The germination of *P. asperata* seeds increases as the duration of each of the freezing stages increases, and in *P. pungens* seeds, the germination of seeds decreases as the freezing intervals increase.

In our opinion, this may be the result of the fact that the level of free water in seed cells of different species has different evaporation rates, which in turn leads to different carbohydrate contents [20].

The most important factors determining the possibility of long-term storage of spruce seeds is their humidity before laying cryopreservation [21]. Comparison of carbohydrate content data in the seeds of the studied species indicates that high carbohydrate content negatively affects the storage process. This contradicts the data available in the literature. Thus, in the work of G.E. Speranza [22] it is indicated that an increase in carbohydrate content contributes to the binding of water molecules, reduces the risk of further dehydration. In pollen studies, a higher carbohydrate content contributed to keeping water within certain limits, resulting in a slower decrease in its viability.

It is found that germination directly related to the type of carbohydrates and their content [17]. This makes it possible to assume that the carbohydrate ratio changes during storage, which requires additional studies.

Conclusion

As a result of the experiments carried out, it was found that the seeds of *P. pungens* and *P. asperata* have a multi-directional reaction to the influence of influences beyond low temperatures. Thus, the energy of germination and germination of *P. pungens* seeds decreases relative to the standard method of stratification of seeds, *P. asperata* seeds increase.

The greatest effect on the safety of *P. pungens* seeds during staged freezing is the time intervals equal to 24 hours, for P. *asperata* seeds — at the time intervals 24 and 168 hours. Accelerated phased freezing at hourly intervals adversely affects the preservation of seeds under cryogenic freezing conditions. Thus, freezing with a time interval of 24 hours should be proposed to freeze the seeds of the two species of spruce being studied.

Correlation between seed germination indices and total carbohydrate content in *P. pungens* and *P. asperata* seeds is determined. It has been established that an increase in the level of carbohydrates in seeds is lithing, exceeding this limit negatively affects the preservation of sowing qualities.

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Л.Б. Арыстан, Т.Х. Камзаева, С.У. Тлеукенова, Д.Ю. Сирман, А.Т. Нуркенова

Шырша тұқымдарының криоконсервациядан кейінгі сақталуы мен өнімділігіне көмірсу деңгейінің әсері

Мақалада *Picea* туысының өкілдері *P. asperata* және *P. pungens* шырша тұқымдарының өміршеңдігіне және сақталуына криоконсервация әдісінің әсері зерттелді. Криомұздатудың баламалы әдісі ретінде температураны кезең-кезеңмен 3 сатылы төмендету әдісі қолданылды: 1 кезең +4 °С, 2 кезең –18 ºС, 3 кезең –196 ºС сұйық азот. Қосымша 4 уақыт аралығының (1 сағат, 24 сағат, 72 сағат, 168 сағат) тұқымның сақталуына әсері зерттелді. Алынған нәтижелерді фотометрия әдісімен түсіндіру үшін 3 мұздату кезеңінің алдында тұқымдардағы көмірсулардың жалпы мөлшері анықталды. Тұқымдардың өнімділік нәтижелеріне жүргізілген талдау зерттелген түрлердің тұқымдары криомұздатуға әр түрлі әсер ететінін көрсетті. Сонымен, *P. asperata* тұқымында 3 эксперименттік нұсқада тұқымның өнімділігі бақылау мәнінен 4–32 % жоғары болды, ал тұқымның жоғары өнімділігі 168 сағат уақыт интервалы қолданылған вариацияда анықталды. *P. pungens* тұқымдарында эксперименттік вариациялардағы тұқымның өнімділік көрсеткіштері бақылау мәндерінен 18–22 % төмен болды, ал эксперименттік вариациялар арасындағы тұқымның жоғары өнімділігі 24 сағат уақыт интервалы қолданылған вариацияда анықталды. Зерттелген тұқымдардағы көмірсулардың жалпы мөлшерін

криомұздатудың әр түрлі аралықтарында анықтау бұл көрсеткіш уақыт аралығына байланысты айтарлықтай өзгеретінін анықтады. Көмірсулардың жалпы құрамының төменгі мәні 24 сағаттық вариацияда, максималды мәні 1 сағаттық вариацияда анықталды. Тұқымның өнімділік көрсеткіштері мен көмірсулардың жалпы мөлшері арасындағы корреляциялық талдау осы көрсеткіштер арасында жоғары байланыс орнатты. Сонымен *P. asperata* шыршасында Пирсон коэффициентінің мәні 0,86, ал *P. pungens* шырша тұқымында 0,91 деңгейінде болды.

Кілт сөздер: криосақтау, тұқым, азот, өнімділік, өсу қарқындылығы, көмірсу, *Picea pungens, Picea asperata.*

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Влияние содержания углеводов в семенах на их всхожесть и сохранность при криоконсервации

Статья посвящена изучению влияния криозамораживания на сохранение посевных качеств семян представителей рода *Picea*, *P. asperata* (ель шершавая) и *P. pungens* (ель колючая). В качестве альтернативного шокового способа криозамораживания был применен метод поэтапного 3-ступенчатого снижения температуры: 1 этап — +4 ºС; 2 — –18 ºС; 3 — –196 ºС. Дополнительно было исследовано воздействие 4-х интервалов (1 ч, 24, 72, 168 ч) ступенчатого замораживания на посевные качества семян. Для объяснения полученных результатов методом фотометрии было определено общее содержание углеводов в семенах перед 3-м этапом замораживания (криоконсервации). Проведенный анализ результатов всхожести семян показал, что семена исследованных видов разнонаправленно реагируют на поэтапное криозамораживание. Так, у семян *P. asperata* в 3-х вариантах всхожесть семян была выше контрольных значений, в пределах 4–32 %, наибольшая всхожесть семян была выявлена в варианте с интервалом 168 ч. У семян *P. pungens* показатели всхожести семян в эксперименте были на 18–22 % ниже контрольных значений, а наибольшая всхожесть семян была обнаружена в варианте с интервалом 24 ч. Определение общего содержания углеводов при различных интервалах криозамораживания установило, что данный показатель значительно варьирует. Наиболее низкие значения общего содержания углеводов были выявлены в вариации с временным интервалом 24 ч. Максимальное значение общего содержания углеводов было определено в вариации с временным интервалом 1 ч. Корреляционный анализ между показателями всхожести семян и общим содержанием углеводов установил высокую взаимосвязь между данными показателями. Так, у *P. asperata* значение коэффициента Пирсона составило 0,86, а у семян *P. pungens* — 0,91.

Ключевые слова: криозамораживание, семена, азот, всхожесть, прорастание, углеводы, *Picea pungens, Picea asperata.*

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