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Study the germination of seeds of Sudanese grass (*Sorghum × drummondii*) treated by ethylene glycol and propylene glycol cryoprotectants

The present study was conducted at an objective study of the seeds of Sudanese grass for cryopreservation and viability of seeds. Laboratory studies were carried out on 4 varieties of Sudanese grass: Nika, Tigai, Novosibirskaya 84, and Alina. The results showed that cryopreservation had a positive effect on germination rates. Thus, in the Tugay variety, the best germination rates of 99 % were observed in the variant with cryopreservation with defrosting of seeds in a water bath. A positive effect was also noted for the variety Novosibirskaya 84, where cryopreservation during defrosting of seeds at room temperature increased germination by 7 % and amounted to 89 %. For varieties Nika and Alina, cryopreservation had the opposite effect, the indicators decreased by 27 %, by 10 %, respectively, compared with control. Under conditions of seed defrosting at room temperature, cryopreservation with ethylene glycol and propylene glycol had a positive effect on the germination of seeds for all varieties, with the exception of the Alina variety, where its germination did not differ significantly from the control. Cryopreservation using ethylene glycol under conditions of seed defrosting at room temperature showed that for the Tugay variety the recommended concentration of the cryoprotectant is 5 % and 10 %. The germination rate was 98 %, and the germination energy was 99 % and 96 %, respectively. Ethylene glycol concentration of 20 % significantly reduced the germination rate and amounted to 47 %. For the Nika variety, we recommend using a concentration of 15 %; but the concentration of 10 %, the germination rate is lower and amounts to 58 %. Almost 100 % germination were observed in the Novosibirskaya 84 variety at concentrations of 5 % and 20 %. Thus, the recommended method for thawing Sudan grass seeds after cryopreservation using ethylene glycol and propylene glycol cryoprotectants is thawing at room temperature. These results can be used to create a cryo collection of Sudan grass seeds.

Keywords: Sudanese grass, seeds, cryoprotectant, plants, ethyleneglycol, propyleneglykol, germination, liquid nitrogen, cryopreservation.

Introduction

Analyzing the climate of Central Kazakhstan, its main features are its sharp continental; deficit of precipitation. Winter is moderately severe with little snow, summer is warm and hot. The cultivation of heat-loving crops is impossible due to lack of moisture. Rains with a small amount of precipitation in summer slightly moisten the soil, so the duration of the dry period increases significantly [1].

One of the promising drought-resistant crops that are suitable for growing in the arid conditions of Kazakhstan in the Karaganda region is Sudanese grass *(Sorghum × drummondii).*

Sudanese grass is an unpretentious and plastic crop with high heat resistance, which in difficult conditions is capable of forming 2–3 mowing of green mass. A distinctive feature of this crop is its exceptionally high drought resistance; it uses well precipitation in the second half of summer and forms a large aboveground mass. Along with this, the culture is distinguished by its high after math ability, good shoot-forming ability, abundant bushiness and rapidity of regrowth. Drought resistance is ensured by a powerful root system that allows using water from deep soil layers [1, 2].

With the introduction of Sudanese grass in culture, one of the important aspects is the preservation of valuable varietal seed material. Seeds are the most optimal form of genetic material storage, as samples require little space and remain viable for a long period. There are several methods for storing plant seed. One of the most inexpensive and effective methods is cryopreservation [3].

The conservation of plant genetic resources is fundamental for the development of agriculture [4]. The pre-planting seeds treatment of agricultural plants with cryoprotectants is accompanied by the improvement of seeds planting characteristics and increasing the crop capacity of treated cultures [5].

Ethylene glycol was found to be a key compound for the successful cryopreservation of shoot tips, in light of its low toxicity as compared to other cryoprotective compounds [4].

Our study was aimed to optimization of cryopreservation conditions using ethylene glycol and propylene glycol cryoprotectants.

Experimental

The objects of the study were Sudanese grass *(Sorghum × drummondii)* seed material, varieties Nika, Tugay, Novosibirskaya-84, Alina (Fig.) and plants sprouts. Seeds were provided by LLP "Scientific and production center of grain farming named after A.I. Baraev" (Shortandy, Kazakhstan).

I — Tugai; II — Nika; III — Novosibirskaya 84; IV — Alina

Figure. Seeds of Sudanese grass

Cryopreservation was carried out by immersing the seeds in plastic cryovials (Deltalab) into Dewar flasks SDS-20 CryoMash with liquid nitrogen (–196 °C). Cryofreezing was carried out in two ways: a) the seeds were immediately immersed in liquid nitrogen without any preparation; b) before immersion in liquid nitrogen, the seeds were treated for 10 min with cryprotectiveethylene glycol and propylene glycol solutions [3, 6, 7].

The prospective types of cryoprotectants were used ethylene glycol and propylene glycol due to the fact that it has high cooling activity [8–10]. Defrosting of seeds were conducted two ways: i) at room temperature ii) in a water bath. To prevent recrystallization of intracellular and extracellular ice in seeds, defrosting was carried out in a water bath at a temperature of +40 °C until complete defrosting up to 15 min [3].

Efficiency of cryopreservation of Sudan grass was determined based on the analysis of seed germination and germination energy [6]. After thawing, the seeds were placed for germination in Petri dishes (diameter 90 mm) of 40 pieces, 4 replications. The germination was carried out in a Binder climatic Chamber at a temperature of +24 °C with constant illumination. Germination was assessed on the 10^{th}day, germination energy on the 5^{th} day. Seeds without treatment served as control 1, cryopreservation of seeds without cryoprotectant. Defrosting at room temperature served was as control 2, cryopreservation of seeds without cryoprotectant. Defrosting in a water bath served as control 3 (Table 1).

Table 1

Abbreviations

Character	Abbreviation
Seeds without treatment	control 1
Cryopreservation of seeds without cryoprotectant. Defrosting at room temperature	control 2
Cryopreservation of seeds without cryoprotectant. Defrosting in a water bath	control 3

Statistical processing of the results of the experiment was carried out according to the Student-Fisher method. Statistical the significance of discrepancies between the values was assessed using Student's t-test.

Differences were considered significant at $p < 0.05$. In the work, 4 concentrations of the cryoprotectants from 5 to 20 % were used.

Results and Discussion

Analyzing the germination rates for varieties among the control, in the Tugay variety the best germination rates of 99 % were observed in the variant with cryopreservation with defrosting of seeds in a water bath (Table 2).

For the Novosibirskaya 84 variety, cryopreservation during seed defrosting at room temperature increased the germination rate by 7 % and amounted to 89 %. For varieties Nika and Alina, cryopreservation significantly reduced germination and germination energy by 27 % and 10 %, respectively, compared to control 2.

Table 2

Germination and germination energy of seed in control variants

Variety	Germination, %	Energy of seed germination, %
Tugai control 1	90 ± 0	95 ± 5.77
Nika control 1	77.50 ± 5	87.50 ± 9.57
Novosibirskaya 84 control 1	82.50 ± 5	90 ± 11.55
Alina control 1	52.50 ± 25	70 ± 18.26
Tugai control 2	90.50±1.29	88.75±2.99
Nika control 2	49.75±1.71	48.25±3.30
Novosibirskaya 84 control 2	89.75±1.71	96.50±3.11
Alina control 2	42.50±9.57	45±5.77
Tugai control 3	99.75±0.50	99.50±1
Nika control 3	76.50±3.87	80.75±2.99
Novosibirskaya 84 control 3	80±2.16	80±3.56
Alina control 3	47.50±9.57	47.50±5

In general, the germination of Sudanese grass in the control is adequate. The next step was to study the germination using ethylene glycol cryoprotectant under defrosting conditions at room temperature.

Cryopreservation using ethylene glycol under conditions of seed defrosting at room temperature showed that for the Tugay variety the recommended concentration of the cryoprotectant is 5 % and 10 %. The germination rate was 98 %, and the germination energy was 99 % and 96 %, respectively. Ethylene glycol concentration of 20 % significantly reduced the germination rate and amounted to 47 %. For the Nika variety, we recommend using a concentration of 15 %; at a concentration of 10 %, the germination rate is lower and amounts to 58 %. Almost 100 % germination in the Novosibirskaya 84 variety at concentrations of 5 % and 20 %.

The use of a cryoprotector significantly accelerated the energy of seed germination, with the exception of the Alina variety, where germination was better in the control and amounted to 70 %, while with the use of a cryoprotector 40 %, 50 % (Table 3).

Table 3

Germination and energy germination of seed treated by ethylene glycol (EG) and defrosting at room temperature

Variety	Germination, %	Energy of seed germination, %
1	2	3
Tugai EG 5 % room t.	98 ± 1.83*	99,75 ± 0,50
Tugai EG 10 % room t.	98.50 ± 1.91*	96,50 ± 3,42
Tugai EG 15 % room t.	90.25 ± 4.92	97,50 ± 2,08
Tugai EG 20 % room t.	47.50 ± 17.08*	99 ± 0,82
Nika EG 5 % room t.	79 ± 3.92	97,25 ± 2,22
Nika EG 10 % room t.	58.50 ± 5.97	99,25 ± 0,96
Nika EG 15 % room t.	90.75 ± 0.96*	95,75 ± 3,50
Nika EG 20 % room t.	81 ± 2.58	97 ± 2,58

Continuation of Table 3

1	2	3
Novosibirskaya 84 EG 5 % room t.	98 ± 2.16*	99,75 ± 0,50
Novosibirskaya 84 EG 10 % room t.	90.50 ± 2.52	98,50 ± 1,91
Novosibirskaya 84 EG 15 % room t.	96.75 ± 2.75*	99,50 ± 1
Novosibirskaya 84 EG 20 % room t.	99.75 ± 0.50*	98,50 ± 1,29
AlinaEG 5 % room t.	40.25±0.73	40,75±0,99
AlinaEG 10 % room t.	50±1.05	40,75±0,55
AlinaEG 15 % room t.	50.50±0.75	60,75±1,71
AlinaEG 20 % room t.	40.75±0.55	50,75±0,55
*Significance of differences P ≤ 0.05 compared with control 1.		

The results showed that cryopreservation with cryoprotectant ethylene glycol during slow thawing at concentrations of 5 %, 10 % in the Tugay variety, 15 % in the Nika variety, 5 %, 15 %, 20 % in the Novosibirskaya 84 variety significantly exceeded the control values of 1, while at a concentration of 20 % in the Tugay variety, cryopreservation significantly reduced the control indicators.

Next, we studied the germination of seeds using an ethylene glycol solution and the rapid thawing of seeds after cryopreservation. The results show that seed germination has decreased compared to control 1 and the room temperature thaw test (Table 4). Compared with the control, the germination of seeds in the Nika variety decreased by 40 %, in the Novosibirskaya 84 variety by 35 %, in the Alina variety by 32 %, in the Tugai variety by 20 %.

Table 4

Germination and Energy of seed germination treated by ethylene glycol (EG) and defrosting in a water bath

Variety	Germination, %	Energy of seed germination, %
Tugai EG 5 % water bath	82.50 ± 17.08	87.50 ± 15
Tugai EG 10 % water bath	85 ± 17.32	87.50 ± 12.58
Tugai EG 15 % water bath	70 ± 8.16*	72.50 ± 5*
Tugai EG 20 % water bath	82.50 ± 5	95 ± 10
Nika EG 5 % water bath	47.50 ± 9.57*	52.50 ± 9.57*
Nika EG 10 % water bath	40 ± 23.09	50 ± 18.26
Nika EG 15 % water bath	37.50 ± 22.17	50 ± 23.09
Nika EG 20 % water bath	62.50 ± 22.17	67.50 ± 18.08
Novosibirskaya 84 EG 5 % water bath	47.50 ± 26.30	55 ± 17.32
Novosibirskaya 84 EG 10 % water bath	70 ± 28.28	75 ± 17.32
Novosibirskaya 84 EG 15 % water bath	77.50 ± 9.57	80 ± 8.16
Novosibirskaya 84 EG 20 % water bath	77.50 ± 17.08	82.50 ± 20.62
Alina EG 5 % water bath	20.75±0.99	30.25±0.73
AlinaEG 10 % water bath	50.75±1.26	60.50±0.33
AlinaEG 15 % water bath	50.25±0.87	50.50±1
AlinaEG 20 % water bath	30.75±0.73	40.50±0.58
*Significance of differences P ≤ 0.05 compared with control 1.		

Cryopreservation of EG during rapid thawing significantly exceeded the control values in the Tugay variety at a concentration of 15 % and decreased in the Nika variety at a concentration of 5 %.

The study of the effect of propylene glycol on seed germination showed an increase in seed germination compared to control 1, with the exception of the Alina variety, where germination decreased by 12–22 % at concentrations of 5 %, 10 %, 20 %. Otherwise, the increase in germination in the Tugai variety is on average 7 %, while at a concentration of 15 %, germination decreased by 21 %.

In varieties Nika and Novosibirskaya 84, a positive effect of cryopreservation was observed in all concentrations. Germination energy in all variants of the experiment is high (Table 5).

Table 5

Germination and Energy of seed germination treated by propylene glycol (PG) and defrosting at room temperature

Variety	Germination, %	Energy of seed germination, %
Tugai PG 5 % room t.	98.50 ± 1.91*	96.25 ± 3.30
Tugai PG 10 % room t.	96.75 ± 2.75*	98.50 ± 1.91
Tugai PG 15 % room t.	69.75 ± 4.65*	97.75 ± 1.71
Tugai PG 20 % room t.	97.75 ± 2.06*	99 ± 2
Nika PG 5 % room t.	90.25 ± 1.26*	98 ± 1.63
Nika PG 10 % room t.	97 ± 2.58*	96.25 ± 3.50
Nika PG 15 % room t.	89.50 ± 4.80	97.75 ± 1.71
Nika PG 20 % room t.	97.75 ± 1.71*	98.75 ± 1.50
Novosibirskaya 84 PG 5 % room t.	96.25 ± 3.50	98.25 ± 1.71
Novosibirskaya 84 PG 10 % room t.	98.25 ± 1.71*	99 ± 1.15
Novosibirskaya 84 PG 15 % room t.	99.25 ± 0.96*	99.50 ± 0.58
Novosibirskaya 84 PG 20 % room t.	97.50 ± 2.08*	98.25 ± 1.26
Alina PG 5 % room t.	40.75±0.55	60±0.82
Alina PG 10 % room t.	30.25±0.29	60±0.47
Alina PG 15 % room t.	50.50±0.58	60±0.47
Alina PG 20 % room t.	40.75±0.96	50.75±0.55
*Significance of differences $P \leq 0.05$ compared with control 1.		

According to the results of table 4, the values are significantly higher than control 1 in the Tugai and Nika varieties at concentrations of 5 %, 10 %, 15 %, in the Novosibirskaya 84 variety at a concentration of 10 %, 15 %, 20 %.

In the next experiment, we observed a decrease in germination to 25 % for the Tugai variety, up to 35 % for the Nika variety, and up to 22 % for the Alina variety. Thus, the cryoprotectant propylene glycol, when defrosted in a water bath, reduces the germination of seeds of the studied varieties (Table 6).

Table 6

Germination and Energy of seed germination treated by propylene glycol (PG) and defrosting in a water bath

Variety	Germination, %	Energy of seed germination, %
Tugai PG 5 % water bath	72.50 ± 12.58	80 ± 8.16
Tugai PG 10 % water bath	77.50 ±9.57	82.50 ± 12.58
Tugai PG 15 % water bath	67.50 ± 15	72.50 ±18.93
Tugai PG 20 % water bath	65 ± 17.32	70 ± 14.14
NikaPG 5 % water bath	35 ± 17.32	45 ± 17.32
NikaPG 10 % water bath	52.50 ± 12.58	57.50 ± 12.58
NikaPG 15 % water bath	42.50 ± 15	47.50 ±9.57*
NikaPG 20 % water bath	50 ± 8.16*	55 ± 5.77*
Novosibirskaya 84 PG 5 % water bath	50 ± 21.60	55 ± 19.15
Novosibirskaya 84 PG 10 % water bath	62.50 ± 17.08	65 ± 12.91
Novosibirskaya 84 PG 15 % water bath	57.50 ± 15	67.50 ± 15
Novosibirskaya 84 PG 20 % water bath	70 ± 21.60	75 ± 17.32
AlinaPG 5 % water bath	50±0.82	50.50±0.75
AlinaPG 10 % water bath	30.75±0.99	40.25±0.87
AlinaPG 15 % water bath	40.25±1.72	40.75±1.36
AlinaPG 20 % water bath	40.50±0.75	50±1.41
*Significance of differences $P \leq 0.05$ compared with control 1.		

According to the results of the table, the performance of the Nika variety was significantly exceeded at a propylene glycol concentration of 20 %.

Conclusion

As a result of the study, the germination of seeds of Sudanese grass of 4 varieties was studied using cryoprotectants ethylene glycol and propylene glycol by cryopreservation. Seeds were thawed in two ways: at room temperature and in a water bath.

Under conditions of seed defrosting at room temperature, cryopreservation with ethylene glycol and propylene glycol had a positive effect on the germination of seeds of all varieties, with the exception of the Alina variety, where its germination did not differ significantly from the control.

Cryopreservation using ethylene glycol under conditions of seed defrosting at room temperature showed that for the Tugay variety the recommended concentration of the cryoprotectant is 5 % and 10 %. The germination rate was 98 %, and the germination energy was 99 % and 96 %, respectively. Ethylene glycol concentration of 20 % significantly reduced the germination rate and amounted to 47 %. For the Nika variety, we recommend using a concentration of 15 %; at a concentration of 10 %, the germination rate is lower and amounts to 58 %. Almost 100 % germination observed in the Novosibirskaya 84 at concentrations of 5 % and 20 %.

The use of a cryoprotector significantly accelerated the energy of seed germination, with the exception of the Alina variety, where the germination rate was better in the control and amounted to 70 %, while with the use of a cryoprotector it was 40 %, 50 %.

Defrosting in a water bath significantly reduces the germination of seeds compared to control 1. When using the cryoprotectant ethylene glycol and rapid thawing of seeds after cryopreservation, the germination of seeds in the Nika variety decreased by 40 %, and in the Novosibirskaya 84 variety by 35 %, in the Alina variety by 32 %, in the Tugai variety by 20 %.

When defrosted in a water bath, cryoprotectant propylene glycol reduces the germination of seeds of the studied varieties. Thus, the Tugai variety has up to 25 %, the Nika variety has up to 35 %, and the Alina variety has up to 22 %.

Thus, the recommended method for thawing Sudan grass seeds after cryopreservation using ethylene glycol and propylene glycol cryoprotectants is thawing at room temperature.

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Этиленгликоль және пропиленгликоль криопротекторларын қолдану кезінде судан шөбінің (*Sorghum* × *drummondii*) тұқымының өнуін зерттеу

Зерттеу судан шөп тұқымдарының өнгіштігіне криоконсервациялау әсерін анықтауға арналған. Зертханалық зерттеулер судан шөбінің 4 сорты арасында жүргізілді. Олар «Ника», «Тугай», «Новосибирская 84», «Алина» сорттары. Нәтижелер «Алина» сортын қоспағанда, криоконсервациялаудың нәтижелері барлық сорттар бойынша тұқымдарының өнуіне оң әсер еткенін көрсетті. Ал «Алина» сортының өнгіштігі бақылаудан айтарлықтай ерекшеленбеді. Сонымен, «Тугай» сортында тұқымдарды су моншасында жібіту арқылы криоконсервацияланған нұсқада 99% ең жақсы өну көрсеткіштері байқалды. Бөлме температурасында тұқымдарды еріту кезінде өнгіштігі 7 %-ға артып, 89 % құраған «Новосибирская 84» сортында да оң әсер байқалды. «Ника» мен «Алина» сорттарына криоконсервация кері әсер етті, көрсеткіштері бақылаумен салыстырғанда 27 %-ға және 10 %-ға төмендеді. Бөлме температурасында тұқымдарды еріту жағдайында этиленгликоль мен пропиленгликольмен криоконсервациялау «Алина» сортын қоспағанда, барлық сорттарда тұқымның өнуіне оң әсер етті, бірақ «Алинаның» өнуі бақылаудан айтарлықтай ерекшеленбеді. Сонымен қатар бөлме температурасында тұқымдарды жібіту жағдайында этиленгликольді қолдана отырып криоконсервациялау «Тугай» сортына криопротектордың ұсынылатын концентрациясы 5% және 10% екенін көрсетті. Өну жылдамдығы 98 %, ал өну энергиясы сәйкесінше 99% және 96% болды. Этиленгликольдің 20% концентрациясы өну жылдамдығын едәуір төмендетіп, 47 % құрады. «Ника» сорты үшін 15 % концентрацияны қолдану ұсынылған, бірақ концентрациясы 10 %, өну жылдамдығы төмен және 58 % құрайды. «Новосибирская 84» сортының 5 % және 20 % концентрациясында 100 %-ға жуық өнуі байқалды. Осылайша, этиленгликоль және пропиленгликоль криопротекторларын қолдана отырып, криоконсервациялаудан кейін судан шөбінің тұқымын ерітудің ұсынылған әдісі бөлме температурасында еріту болып табылады. Бұл нәтижелер судан шөбінің тұқымдарының криогендік коллекциясын жасау үшін пайдаланылуы мүмкін.

Кілт сөздер: судан шөбі, өсімдіктер, этиленгликоль, пропиленгликоль, сұйықазот, криоконсервация.

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Изучение всхожести семян суданской травы (*Sorghum* × *drummondii*) при использовании криопротекторов этиленгликоля и пропиленгликоля

Настоящее исследование посвящено изучению влияния криоконсервации на всхожесть семян суданской травы. Лабораторные исследования проводились на 4 сортах суданской травы: «Ника», «Тугай», «Новосибирская 84», «Алина». Результаты показали, что криоконсервация положительно повлияла на всхожесть семян всех сортов, за исключением сорта «Алина», где её всхожесть значительно не отличалась от контроля. Так, у сорта «Тугай» наилучшие показатели всхожести 99 % наблюдались в варианте с криоконсервацией с размораживанием семян на водяной бане. Положительный эффект отмечен и для сорта «Новосибирская 84», где криоконсервация при разморозке семян при комнатной температуре увеличила всхожесть на 7 % и составила 89 %. У сортов «Ника» и «Алина» криоконсервация дала обратный эффект, показатели снизились на 27 и 10 % соответственно по сравнению с контрольной группой. В условиях разморозки семян при комнатной температуре криоконсервация этиленгликолем и пропиленгликолем оказала положительное влияние на прорастание семян у всех сортов, за исключением сорта «Алина», где его прорастание существенно не отличалось от в контрольной группе. Криоконсервация с использованием этиленгликоля в условиях разморозки семян при комнатной температуре показала, что для сорта «Тугай» рекомендуемая концентрация криопротектора составляет 5 и 10 %. Скорость прорастания составляла 98 %, а энергия прорастания — 99 и 96 % соответственно. Концентрация этиленгликоля 20 % значительно снижала скорость прорастания и составляла 47 %. Для сорта «Ника» рекомендуем использовать концентрацию 15 %; но концентрация 10 %, скорость прорастания ниже и составляет 58 %. Почти 100 % всхожесть наблюдалась у сорта «Новосибирская 84» в концентрациях 5 и 20 %. Таким образом, рекомендуемый способ оттаивания семян суданской травы после криоконсервации с использованием криопротекторов этиленгликоля и пропиленгликоля является оттаиванием при комнатной температуре. Эти результаты могут быть использованы для создания криогенной коллекции семян суданской травы.

Ключевые слова: суданская трава, растения, этиленгликоль, пропиленгликоль, жидкий азот, криоконсервация.

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